0273-2289/00/83/0297/$14.25

General Discussion II

Chaired by S. Paul

Paul: Perhaps we can start with the issue of the functional role of catalytic antibodies in innate and adaptive immunity and the pathological role in autoimmune disease. Yesterday, definitive experiments were suggested, upon which the health of the field in dependent. Those are good and clean experiments. We don't have to go over the details, but are these experiments worthwhile? We can make transgenic mice by overexpressing the catalyst, or we can ablate the catalytic antibody genes, but these are time-consuming experiments costing millions of dollars. Are these experiments worth doing?

Shoenfeld: Not being really involved with catalytic autoantibodies per se, I have the fear that maybe it is just an epiphenomenon, and we have no evidence that they are really involved in pathogenesis. So what I would like to see in the next meeting is more evidence for the involvement in autoimmune diseases; for example, a relationship to clinical manifestations. Yesterday, Noel Rose said he was sorry that he didn't send antibodies to Sudhir from first-degree relatives of thyroiditis patients who don't have symptoms, but may have autoantibodies that may or may not have catalytic activities. I would like to see that if you isolate the antibodies, you are able to induce a disease in comparison to a noncatalytic antibody. Then, I would like to have a reasonable explanation for the involvement in pathogenicity.

Paul: We do not want to get lost in a laundry list of evidence required to make a molecule firmly linked with disease. Your field—the anti-Id field—is far more developed than the catalytic antibody field, but is viewed with great skepticism. This does not mean that anti-Ids are meaningless molecules. You pose good challenges, but we need to maintain some balance that life processes are multifactorial processes, involving anti-Ids, the catalytic activity, the enzymes, the beneficial antibodies—all of these come together in homeostasis. To respond specifically, statistical study of the catalytic activity and the disease process has been initiated. However, even after we correlate the clinical symptoms with the catalytic titer, I admit there will still be questions about whether catalytic antibodies are important mechanistically. Therefore, we want to use a catalyst to induce a disease in a mouse and to ablate the disease with inhibitors.

Kohler: I think there's a cheaper way than the transgenic catalytic mouse. You just do passive transfer of a purified catalytic Bence Jones in a mouse and see what happens. Or you transplant a hybridoma tumor in the mouse and see what happens. You get a very high titer of the catalytic antibody. You may not have enough time to observe the mouse, but you at least get an idea whether something is wrong.

Rose: I think Heinz's experiment is a great experiment, except what in the world do you look for? I'm not sure what you are fishing for. I think I presided over the demise of more fishing expeditions than anybody in this room.

Paul: The target defines what you look for. In the case of anti-VIP, you look for effects that are related to VIP. In the case of thyroglobulin, you focus on the thyroid. In the case of DNA—lupus.

Rose: The statistical relationship between catalytic antibodies and autoimmune disease should be studied in more detail. It is not enough to show that the catalytic activity is greater in patients than in healthy controls. Additional patient controls are needed. I think Yehuda will agree, you compare patients with patients. You don't compare patients with normal individuals.

Shoenfeld: Diseases like lupus have more active stages and then nonactive stages. Correlations between titers and disease stage are needed. We need more statistical data. If you read some of the abstracts in this meeting, you have already jumped into inhibition of catalytic antibodies as a means of treatment of diseases, but we don't know which diseases you would like to treat. First we have to show that the antibodies are really pathogenic. Now, even if they are not pathogenic, they may be important in diagnosing autoimmune disease if there is a good correlation statistically.

Paul: The animal-model inhibition experiment would define very clearly the pathophysiological role of the catalyst. Whereas statistics would establish only the association, not necessarily the role.

Brahmachari: I just don't know how sure you can be at the end of the animal experiment, because compensatory events can happen. After the antibody cleaves and destroys the substrate molecule, the pathway for synthesis of substrate may be upregulated. Then you'll find nothing. This has been the fate of several transcription factors. We wanted to prove that the transcription factors are decisively involved in the disease process, but it turned out that the mice are happily moving around even when you knock out what you thought would be the most essential transcription factor.

Neuberger: There is obviously much redundancy. If you knock one thing out, something else will come in. The opposite problem in the models of catalytic antibodies is that the antibody may be part of the pathology, but it may not be sufficient to cause it. Therefore, even if the so-called defini-

tive experiments fail, they do not invalidate the importance of catalytic antibodies. You can say you just need other contributing factors.

Rose: I think one has to face the reality that a positive experiment might be interesting, but a negative experiment really doesn't inform us at all.

Zouali: Yes. I'm working on catalytic antibodies, but I also run a gene-targeting facility. We made more than 50 knockouts in the last few years, and I am quite involved with the analysis of those animals. In some cases you can get a definitive answer, but in most cases you end with more questions than you had when you started. So before one goes into either transgenic or knockout mice, it's probably more beneficial to gather more evidence from other studies because it is very complex to analyze the full animal.

Rose: The problem with knockout mice is that they survive.

Shoenfeld: When people wanted to show that idiotypes of anti-DNA antibodies are pathogenic, they generated transgenic mice expressing the idiotype, but they were not secreted into the serum and the model was worthless. Sudhir, you showed us prothrombin-cleaving antibodies. I wanted to caution you that Noel Rose and myself now have in Japan a beta2-glycoprotein knockout mouse that is quite happy and has not developed any coagulation syndrome.

Brachmachari: We have only 25,000 proteins floating in our blood, and 80,000 proteins total. Now we are talking about millions of additional proteins—the antibodies—and it is most likely that they will have additional functions, because they have all the charges, hydrophobic interaction capabilities, and proton- and electron-transfer capabilities.

Paul: Let me try to show the importance of antibody catalysis with the following question. How many nonantibody catalysts do we know that are functionless—that have no biological function?

Kirby: You should say "no known function."

Paul: Right. Do we know of any biological catalysts that have no known biological functions?

Marchalonis: Sudhir, remember the history of enzymology. Activities were detected and biological function was not known. You could assay color changes, oxidations, cleavages. It took years to establish what the enzyme was doing in a particular pathway. So, in a sense, maybe we are expecting too much in too short a time period.

Paul: Professor Kirby, I thought you were going to say it depends on the level of catalytic activity.

Kirby: No, I was going to say it's not a helpful question. It is a dead-end question because of the problem of currently unknown functions.

Paul: My point, in fact, was the opposite. I cannot think of any biological molecule that is a known catalyst but does not have a biological function. I exclude low-activity molecules like albumin.

Green: Before we leave this topic, I wanted to draw the analogy between ribozymes and catalytic antibodies, in the sense that they started as curiosities that nobody believed in and have now developed into a rich career.

Neuberger: Ribozymes actually started out as having a natural function, the self-splicing RNAs—they started with the biology behind them.

Green: I'm not an expert in the field, but RNA self-splicing at that time was as speculative or more speculative than the proposed biological role of catalytic autoantibodies is now.

Neuberger: I think the analogy is OK. But let's go on to the question of whether there is a role in biotechnology applications. That is probably where the money is. Sutton's law: you go to where the money is. That's why people rob banks.

Kirby: I will comment at a fairly superficial level. I was at a physical chemistry meeting on catalysts last year, where I was at the thin end of the wedge. What chemists and industry want are robust and efficient catalysts with clear applications that will do things that industries need. Now that is something we have not really been talking about, but the biotechnology connection is clearly a live one. I come back to my point that until catalytic antibodies become designably efficient, they don't have a role in the high-reward area of industry.

Paul: Do you think there is any possibility that they will be made both unique and efficient?

Kirby: As I said yesterday, the encouraging thing has been the high activity of the DNA-cleaving antibodies from autoimmune patients. If we could get clean molecules and learn from those molecules, then I'm most encouraged by what I've heard in this meeting. But the important point is that the targets that industry is interested in are unique target reactions that enzymes can do.

Tramontano: So you want a unique function as well as an efficient function.

Vijayalakshmi: If we can develop a diagnostic test through a catalytic antibody for autoimmune disease, there will money. Just one single test, and money will flow in.

Green: Regarding what Tony Kirby mentioned, I think at the next meeting we would like to know much more about how these antibodies work. When enzymes were discovered, that was a tremendous stimulation for chemists to imitate the enzymes. The chemical community has tried hundreds, if not thousands, of examples of trying to mimic enzymes. I'd like to quote

from the 1902 Nobel lecture of Emil Fisher, who is known for his lock-and-key explanation for enzymes. He said he foresees the time when we will be able to synthesize enzymes. But virtually no catalytic antibody has even remotely approached what enzymes can do. We're unhappy in a sense—Tony's slide showed us how poor the catalytic antibody activities look compared to enzymes. On the other hand, the level of effort in catalytic antibodies has not been tremendous. We have to understand the mechanism of the antibodies, and the fact that there are antibodies that display very efficient peptide-hydrolyzing activity is very encouraging. Maybe this will allows us to design protease antibodies. I also think this field is very young, and many questions remain. The field is driven largely by enthusiasm and subjective beliefs. Many people who entered this field just thought that if an antibody could bind virtually anything, it must bind some structure that will lead to catalysis.

Paul: But fanaticism is hard to sustain without dollars. Buddy, can you comment on a possible happy marriage between the autoimmune repertoire and the biotechnological applications? Is there a way to use the autoimmune repertoire for biotech applications?

Green: Sometimes I feel that with fanaticism you can survive even without dollars. Take my own case. Sudhir is referring to a very surprising observation. When we were looking for other sources of catalysts to expand the repertoire, the suggestion was made that we might try autoimmune-prone mice. I showed you that about 1% of the clones from regular mice were catalytically active. When we used SJL autoimmune-prone mice, virtually all of the clones lit up. So we repeated the experiment and again, the same thing happened. So we jumped from about 1% catalytically active clones to about 75–85% catalytic clones. Now, this benzyl phosphonate immunogen is totally irrelevant to the mouse. The catalytic activity of the antibodies was not really greater than the activity we were getting in the BALB/c mice. One of our goals was to get peptide-hydrolyzing antibodies, and none of those antibodies did this. Just to end the story, if we do a short immunization period, we get frequent catalysts. After a long immunization of the same mice, the proportion of catalytic clones fell dramatically to maybe 10%.

Schowen: There is a certain amount of growing evidence that esters with very reactive leaving groups, like nitrophenol, are not hydrolyzed by the traditional tetrahedral intermediate mechanism, but by some mechanism in which the leaving group breaks off simultaneously with the attack of a nucleophile. I don't think anybody really knows whether the reaction goes through a tetrahedral-like transition state or some totally different structure of the transition state. But I wonder if some part of the catalytic activity of your very good antibody might come from the fact that you used a benzylester substrate, which would probably promote tetrahedral character in the transition state, unlike the para-nitrophenol esters?

Green: One antibody also hydrolyzes para-nitro phenol esters. catELISA could not be used with the para-nitrophenol substrate because it's too labile, giving too much background reaction. You are absolutely correct. To get a good catalytic antibody from the catELISA, it is better to study substrates somewhat similar to the transition-state analog. A family of catalytic antibodies of which Marcel Knossow has very nice crystal structures are specifically raised against a para-nitrophenyl phosphonate, and it would be interesting whether one could explain the reaction along those lines.

Christen: Can you give a sense of how much more sensitive your catELISA is to direct analysis of the product—say, by a photometric method? You measured the activity of the marker enzyme on the second antibody, which will increase sensitivity. On the other hand, you have an immobilized substrate, which might slow the reaction.

Green: I don't know the sensitivity, but it's extremely sensitive. The approach could be modified to improve it further. One could use a biotin system. We can pick up catalysts that have rate enhancements of only 700. You would not see such weak catalysts by any other method.

Zouali: Have other groups also done direct screening for catalysis?

Green: Richard Lerner and others have proposed systems for direct screening, but they've never been used to actually discover a catalytic antibody to the best of my knowledge.

Tramontano: I wonder if single turnover antibodies bind to the immobilized product in the catELISA plates, and this leads to selection against product inhibition. Product binding without catalysis will mask the product from detection with the antiproduct antibodies, and the wells will be classified negatives.

Green: In a sense that is what we have tried to do. I have the feeling that these antibody pockets are quite similar. It seems we used a smidgen of the huge repertoire. When I first spoke with the antibody people like Michael Sela, I had this grand view of hundreds of millions of possible antibodies in our response, with each antibody being a totally different molecule. But there are only minor amino-acid differences in three different catalytic antibodies. I think it will be interesting to consider what type of generic pockets are capable of catalysis. I think what you suggested would put us back into that same kind of pocket. Incidentally, our pockets are very similar to antibodies that simply bind haptens. Antiarsonate or antiphosphocholine antibodies have overlapping product sequences with the catalysts. We generate an acetate product in this system, which is probably not going to be inhibitory. The catELISA is designed to bind to the aromatic group. Any antibody that binds too tightly to the aromatic group will not be picked up.

Tramontano: From the crystal structure, is it clear which groups in the site contribute to protonation of the leaving group?

Green: We don't have protonation of the leaving group here because it is the alkoxide that is presumably leaving.

Tramontano: When you saw the structure of the benzyl alcohol in the antibody, it was hydrogen bonded to the asparagine, which could be part of a proton relay.

Green: Asparagine is just hydrogen bonded to the hydroxyl. I think things like this have been invoked previously, but it is speculative.

Tramontano: So you are thinking now about engineering—general bases in the vicinity of the bound substrate.

Green: That will be an interesting approach. I am not a site-directed mutagenesis man. What I would like to do is make different hapten immunogens and then hope to tap into a broader antibody repertoire. We think the repertoire has been very restricted thus far in the transition-state analog-elicited catalytic antibodies. You are alluding, I am sure, to the possibility of peptide-bond hydrolysis—if we could put something in the catalyst that would protonate the leaving group.

Tramontano: The other difficulty in the designer approach is that no one has been able to design an immunogen analog that can invoke multifunctional catalysis with general acids and bases.

Green: You are quite right. There are strengths and weaknesses in this approach. The strength is that we can get powerful catalysts for ester hydrolysis. I would go so far as to say that there is probably no ester we could not hydrolyze using a catalytic antibody, especially if it had an aromatic group somewhere. And there are biotech applications—for example, hydrolysis of toxins that have esters. But the weakness is that we have no chemical catalysts—this is only transition-state stabilization.

Kohler: Would the dependency on hydrophobic pockets in your approach actually be a problem in finding catalytic antibodies that can cleave the peptide bond in a large protein?

Green: I am a great fan of catalytic antibodies, as everybody who knows me will attest. But there are problems, and we need further developmental work on catalytic antibodies. The antibody system is very promising, as was pointed out by Daniel, as a system for therapeutics. The IgG has a half-life of about 3 wk in the human being, so that is wonderful. Concerning how to go on further, we might use the principles we've learned from crystal structures to go to other systems. Over the years, Tony Kirby and I have discussed possibilities like moving back to the enzyme models. Another possibility is to use nonprotein frameworks for catalysis. There are plastic antibodies—plastibodies or imprinted polymers. The idea is that you polymerize around a hapten, then you extract your hapten and there is a

cavity capable of nice catalysis. Some of the lessons from catalytic antibodies could be applied to those systems. At any rate, I think there are a lot of cross-fertilization possibilities. The problem of our sampling such a tiny domain of the immune space—it was very sobering to me, and I think a lot about how can I overcome this. The phage display system was mentioned as one approach to get more diversity.

Tramontano: About this notion of a chemist not being able do as well as nature—I think this is incorrect. Everyday pharmaceutical chemists are doing chemical steps in a more efficient way using enantioselective catalysts, cobalt catalysis, transition-metal catalysis. These are important for biotechnology applications and for pharmaceutical applications, and the catalysts are as good or better than natural catalysts. These are all just unnatural catalysts.

Green: I certainly agree. As a chemist, I vote for chemistry.

Tramontano: Concerning the connection to autoimmunity, perhaps the connection is really an epiphenomenon. The catalytic activity directed toward a specific antigen is an abnormality, but there may be an important function for catalysis in a healthy individual relating to the ability of the B cell to regulate the immune response by destroying the antigen before it can be presented to the T cell. The B cell is not just an antibody-producing cell, it is also an antigen-presenting cell. As the B cell matures, it recruits T-cell help. It presents the T-cell epitope to a specific T-cell clone and then gets the requisite cytokines and proliferates. But if the B cell makes a catalytic light chain, which is active within the cell, it will destroy the antigen before it can be taken up by the MHC antigens. Therefore, the B cell will no longer present the T-cell epitope, and will be downregulated.

Rose: So the catalytic activity will lead to a nonresponse rather than a response. The B cell will present antigens in ways that get a signal to proliferate. It does not present so that the T cell proliferates. The T cell gets its original stimulus from other professional antigen-presenting cells. The role that you are proposing for the B cell is not a conventional role.

Tramontano: I was suggesting that the B-cell antigen-presenting function is most important when it matures along the affinity-maturation process. As it reaches the point where the affinity maturation is complete, the cell will be downregulated. I think that the immune system would like to stop affinity maturation and not produce a higher and higher affinity for its antibodies. In regard to autoimmunity, the catalytic destruction of an antigen may just reflect a dysregulation of the presentation pathway.

Rose: But that is a response to the autoimmunity, not the cause of the autoimmunity. That follows from your hypothesis.

Marchalonis: This is a somewhat related speculation. We and others found a few years ago that certain antibody subpopulations can specifically bind

certain peptides. The peptides we've looked at are from old albumin and T-cell receptors. So there are antibodies with constitutive specificity for certain peptides. How are these peptides produced from their parent proteins? Because the cleavages are not predicted based upon traditional enzyme specificity, you can test whether catalytic antibodies could generate these peptides, which can then go on to either prime or suppress B cells and T cells.

Paul: If the cleavages are at the appropriate residues, peptides that are bound efficiently by MHC class 2 antigens will be generated. On the other hand, if the cleavages are at the wrong positions, then peptides that are not bindable by MHC antigens will be generated. So you can either get an enhancement or a suppression of presentation.

Marchalonis: That's right. It is common knowledge now that T-cell receptors essentially see linear peptides presented in stretched-out form by MHC. In native immunoglobulin-isolated serum, peptides bound in the combining sites of immunoglobulins are found. Where do the bound peptides come from? What is the mechanism for generating them? All this may be tied up in one piece to antibody catalysis. It's a speculation, but it follows from a real phenomenon.

Neuberger: In my opinion, the purpose of the immune response is to generate B cells that will continue to recognize, bind, and respond to the antigen to which they naturally responded. That response is dependent upon immunoglobulin crosslinking, which requires antigen binding. Having initiated that process and recruited T-cell help, if they then lose the ability to bind antigen or the ability to present the antigen to the T cells, B cells will die. They will be killed. Having initiated the process and selected out those cells that are most appropriate, the end result should be to try and keep them alive. I think it is somewhat counterintuitive to suggest that the catalytic activity would result in their death, and that this effect is beneficial.

Paul: At the terminal stages of clonal selection, death can be beneficial. Programmed death might, in fact, be the mechanism to terminate the process of affinity maturation, once the antibody has acquired a sufficiently great binding affinity.

Paul: Concerning simple antigen binding by antibodies, I will put forward a hypothesis for your comments. If the ligand-binding specificity is partly encoded by the germline genes—that is, the germline genes already are specialized to recognize certain antigens, the frameworks might play as dominant a role as the CDRs in the binding specificity. However, the frameworks are likely to have a lesser role in molecules in which adaptive mechanisms are the main determinants of specificity.

Capra: In fact, this is the only way you can explain the existence in most species (not all) of multiple germline V genes. There must be some advan-

tage to carrying around those frameworks, otherwise the immune system could do it all with somatic mutations and a single germline gene. Given enough effort, though, it can all be done with somatic mutations, because in some experiments that we and others have done, you could make almost anything starting with just a couple of light-chain genes if you let the system work at it. But if you want a quick response, then you have to encode the specificity in the germline.

Rose: The cold agglutinins are funny autoantibodies—they are both cold-dependent and IgMs. But the other autoantibody specificities you mentioned are neither. Can the same framework be involved in ordinary IgG antibodies like antiRho autoantibodies, which are not cold-dependent antibodies?

Capra: I don't know if anyone has looked to see if framework 1 is essential for those other activities like anti-DNA and antiRho binding.

Rose: If you were to put the correct framework in to another antibody, would it become a cold agglutinin?

Capra: Yes, putting the framework 1 in to antiarsonate antibodies makes about two-thirds of the antibodies cold agglutinins. There is also a role for structures in CDR3 for the binding, but it is not a big role.

Zouali: Does the cold agglutinin antibody never show up in the circulation?

Capra: It does, but at extremely low levels.

Zouali: If the cold agglutinin is a part of a network, there must be some antiidiotype in the patients. We are now beginning to understand how antiidiotypes induce apoptosis. Is there any evidence of apoptosis?

Capra: I have not really looked, but there are reports demonstrating that the antibodies do have a profound effect on cells. They punch holes in cells, in a way that is not an apoptotic type of death. The cells appear very unusual after treatment with cold agglutinins as seen by electron microscopy.

Koengten: I have a question about the logic of the positive and negative selection of the V genes. The V gene is apparently represented normally at a certain stage of the immune response, but is overrepresented in the naive repertoire in the tonsil. What about the representation in the periphery?

Capra: In the peripheral blood B-cell repertoire, the gene is also represented at a pretty high level. But the antibody never switches to an IgG. It stays at the IgM level in peripheral blood.

Koengten: How do you know there is progression of the cells through the different compartments? Do cells start in one compartment and proceed to the next compartments, or are they really at a dead end within a particular compartment? The cells could be selected for a particular function, and the selection might not allow the cells to proceed through to the next stages.

Capra: We have operated under the assumption that the steps are progressive, but it is probably more complicated. There are probably dead ends and also recirculations of the cells to prior developmental stages. Indeed, we and others have looked at some of the transitions. Usually the progression is stepwise, and we can cross the transitions using the usual potions like T cells and CD40 ligands, but not in one giant step.

Paul: Do we know a way to recruit a specific germline gene encoding the catalytic activity for synthesis of antibodies directly against the desired target antigen? This question might hinge on whether we can happily marry the designer and the autoimmune approach to raise unique and efficient catalysts.

Kohler: Do you want to simply recruit the gene and avoid affinity maturation?

Paul: Recruit the gene and, if possible, improve catalysis over clonal maturation; but at least recruit the gene.

Kohler: Let's just stick with recruitment. The only way you can do that is with B-cell superantigens. The superantigen will not drive affinity maturation.

Zouali: I don't know whether superantigens can or cannot drive affinity maturation in the B cells.

Paul: Just recruitment then, Moncef, of a specific germline gene. Can we coinject our immunogen, which gives us a catalytic response, along with a superantigen? Should we combine the two for immunizations of mice?

Zouali: You could try, but I am not sure of the outcome until you try.

Koentgen: Sudhir, I think you are trying to reinvent our patent. We are injecting a protein that would allow B cells with catalytic antibodies on the surface to proliferate. I don't know where the idea with the germline genes comes from, but our technique should allow for affinity maturation for greater catalysis.

Paul: We know that biased usage of germline genes can occur in autoimmune disease and in responses to microbial infections. What are the factors driving the biased usage?

Zouali: Well, I cannot agree outright with this statement. There is no real bias in the usage of V_H gene in autoimmune disease. What we know is that there is an overlap in the usage of V_H genes by autoantibodies and in antibodies to pathogens.

Paul: It seems we do not know how to recruit germline genes, and we do not know how they are recruited in nature.

Green: Returning to the issue of choosing targets for our catalysts, Al Tramontano and Dan Landry have discussed the use of antibodies for the breakdown on cocaine.

Neuberger: I suggest sickle-cell hemoglobin as a target. There are already antibodies that recognize a single mutation at position six. If you can digest the sickle cell, the patient will do better.

Green: We don't know how to design any peptide-bond breakage. The tools that are available to you today allow you to define a target for tomorrow. One of the activities for the people in this field is to discover new tools. We should have two levels of targets. One level is for targets for which we don't have the tools and on the second level, we may have the tools, but the tools are not good enough. We have to improve the tools in any event.

Paul: I agree in part. Remember, the immune system already provides design at the level of peptide epitopes recognized by antibodies. Within the epitope, we cannot target bonds for cleavage by the antibodies. We just accept what nature give us. But Al, Gennady, and I are selecting for flanking-residue specificity, which is the beginning of our designer efforts.

Neuberger: We've had two very nice ideas. One to break down cocaine and the other of an antisickle antibody. I think we ought to distinguish those two applications. We already have a very challenging goal—to make a catalytic antibody to do catalysis efficiently, breaking down cocaine. We should be careful to not take on an impossibly difficult target requiring efficient delivery of an antibody into the intracellular compartment, which is what the sickle-cell treatment requires. On an unrelated point, I wonder if you could bring the antibody-combining site closer and closer to an enzymatic site, so that binding happens at one end of a molecule containing the antibody combining site and the catalytic activity happens at the other end—and the binding site and catalytic activity become coupled.

Paul: This is the basis of the ADEPT technology that Buddy mentioned. Your other important point was that preferably targets should be extracellular.

Tramontano: Michael's model is consistent with your model of a catalytic antibody-active site with a binding site and a catalytic subsite somewhat remote from each other.

Paul: Yes. We think we already have in an antibody what you are asking for in an enzyme–antibody fusion protein.

Neuberger: Exactly—but what I would like to do is bring them closer and closer together so I'm actually recruiting the active site of the enzyme.

Paul: Regardless of where the catalytic funtionality comes from—enzyme or antibody—how can it be integrated with the initial antigen binding function?

Kirby: I myself am very interested by the idea of tacking the two together. This can work extremely well if you want to cleave DNA, because of the binding-part precision, for instance, with six nucleotides in a row. Cleav-

age can then be done even using rather nonspecific chemical cleavage reagents, but people are gradually focusing on the phosphorous–oxygen cleavage, which is the clean way to cleave DNA. Perhaps this could be done by a catalytic antibody without attaching an enzyme to it. You can attach some nucleotides to the antibody CDRs which would bind, and you can cleave DNA surgically.

Tramontano: The physical–chemical problem with that system is that if you get too high a binding affinity for the substrate—which is also the product in this case—the antibody is not really a catalyst, but just a reagent that clips and then dies.

Paul: That brings us to the issue of transition-state and ground-state binding. Again, I'm not seeking ideas about to how to elicit catalytic antibodies, but just the mechanism of catalysis. Can good catalysis happen solely by transition-state binding? Must we combine good ground-state binding along with good transition-state binding to get the ideal, specific, magic bullets to cure AIDS, cancer, sickle cell anemia, and other diseases?

Kirby: Well, you know that there is a simple answer. You do not want very strong substrate binding. Enzymes typically operate at about millimolar K_m. Of course, that is the general level of concentration of their substrate under physiological conditions—but, nevertheless, that is guidance for us.

Paul: My counter question is: Are enzymes a good model to predict what is required from an antibody? After all, the target antigen concentration is often in the nano- or picomolar range.

Kirby: I would submit that they are the best model that we have.

Paul: That certainly is true. But it is reasonable to start thinking of differences between enzymes and antibodies based on structural data.

Kirby: What enzymes do remarkably well—and catalytic antibodies don't—is to use functional groups for catalysis. Something like half or even more of the catalytic antibodies in literature do not use a functional group—phosphonate-elicited catalytic antibodies, for example.

Paul: I agree. But natural proteolytic antibodies appear to use covalent catalysis mechanisms involving the chemistry of functional groups.

Shoenfeld: If so, selection for simple binding by antigen is the wrong way.

Marchalonis: The other thing we can do is to build upon the available properties of antibodies. One of the big debates 30 years ago was whether the specificity resides in the heavy chain or in the light chain. It turned out, in most cases, that it resides in the interaction of both chains. Just taking the cards that were dealt to us, we could set up different models and see what happens. One model is an antibody where the specificity resides in the V_H. Then you just make a heterodimer of the V_H and the V_L with the catalytic

activity. Put the two of them together, and we've done essentially what you've recommended, Mike. We have the two halves with different activities in one combining site. It's either going to work or it's not. It will probably work in some cases and not in others. This is really based upon our knowledge of immunoglobulin structure that was obtained from 30 years of struggle and controversy, so we may as well use it.

Paul: I agree that it is unwise to lose what antibodies have already gained naturally—namely, high-affinity binding for the ground state. In the quest for better transition-state binding, I think it is wise to seek ways of combining the two events: the ground state and the transition-state binding.

Neuberger: A plea for compromise. I came along here knowing very little about catalytic antibodies, but I assumed that only very efficient selection would work to isolate catalysts. Having heard all, I now plead for compromise. Starting to bias the repertoire toward catalytic activity, as we've heard, might help; trying to select for antigen binding but putting the bar somewhat low might help—finally the weak binders are subjected to stringent selection for catalysis. If you have a fantastically robust, uniformly applicable selection for catalysis, then you can just do it from random repertoires and we wouldn't be discussing this. But I wonder if the answer will be in a combination selection.

Zouali: Dr. FitzGerald, can you give your perspective on how phage display compares with the in vivo methods based on inserting the human immunoglobulin genes into the mouse genome?

FitzGerald: Phage display is a more direct approach to getting antibodies to specific epitopes. Also, it's a lot quicker. You get antibodies of the desired affinity and specificity in a week where you obviously need fairly lengthy protocols by the hybridoma approach. The third winning feature, in my view, is the capacity. There is absolutely no way the conventional immunization protocol can possibly yield antibodies to 98 antigens in a week, as is possible in the phage protocol.

Kohler: In antibody therapy, the problem is not that we fail to find new antibodies against new targets. The problem is that we do not find more effective antibodies. If you just look for antibody-staining intensity in different tissues, this does not predict what will happen in therapy. In vitro tests—like complement of ADCC—also do not predict how these antibodies will work therapeutically. So my question is: how do you find the effective antibodies from your library?

FitzGerald: At CAT, we have a team dedicated to antibody engineering, which allows us to modify affinities and specificities to a greater degree than ever before. We were able to generate antibodies with affinities in the picomolar range. We can reclone these genes into vectors to reconstitute an IgG with any isotype we wish. These technologies simply increase the total supply of leads for therapeutics discovery. If we can do activity

assays in high throughput, we are more likely to get antibodies that have therapeutic value.

Paul: Is there a distinction between affinity and specificity? Are high-affinity binders often cross-reactive, or can we assume they are quite specific?

FitzGerald: Well, we have produced many antibodies which aren't very high affinity, but are specific. These can be affinity matured. I gave you the example of hemoglobin binders that reacted specifically to the fetal, but not the adult, hemoglobins. The original affinity of that clone was in the micromolar range.

Paul: Let me ask you a question in a different way. Do you have antibodies that have K_D values in the nanomolar range—and yet are nonspecific and cross-react with other antigens? Conversely, do you have antibodies that are highly specific and display micromolar K_D values?

FitzGerald: Well, yes. That's true in the example of the hemoglobin binding with K_D in the micromolar range, but the antibody is very specific for the fetal and not the adult hemoglobulin. I can't answer the other question—whether we have very high-affinity binders that are nonspecific.

Paul: A related question is: What is the antigen-binding affinity of these germline-encoded antibodies? Are they relatively nonspecific?

FitzGerald: We have isolated high-affinity antibodies to a number of targets that have accumulated sequence changes during the affinity maturation process. We were able to convert those sequences back to germline without losing the affinity.

Green: What about phage antibodies as catalysts? When the phage technology was first developed, we thought that would be the end of hybridoma technology. Yet in practice, there are no spectacular examples of new catalysts or greatly enhanced activity.

FitzGerald: When I joined CAT, the first project I worked on was getting antibodies to a transition-state analog. We did generate a large number of both human and murine antibodies by phage display to the transition-state analog, but unfortunately, none of them were catalytic.

Paul: There are many here who are interested in exploiting the phage technology. Yes, there are substantial technological hurdles to be crossed, including doing phage-catalysis assays as opposed to binding assays, the issue of contaminants, and the issue of expression level on the phage surface and in soluble form.

Zouali: The title of the meeting is "Catalytic and Superantibodies." Before coming here, one of my colleagues asked me: what is a superantibody? My response was: I'll tell you when I come back. So before going back to Paris, I would like to have a precise definition of what a superantibody is.

Kohler: Sudhir and I struggled with the term "superantibody" quite a bit. I think there is probably a reasonable definition today if you accept that it still a developing concept. So what we say today might need to be modified somewhat later. Sudhir and I tried to define superantibodies as a special class of antibodies. The distinction is that an ordinary antibody is defined by its antigen binding and F_C-mediated effector mechanisms. The special class of superantibodies can occur in nature, and they can also be engineered. Superantibodies have biological activities that are not necessarily mediated by the CDRs. In structural terms, superantibody activities can be expressed by the framework structures as well by CDR structures. I tried to emphasize this by saying this is a happy marriage between innate and adaptive immunity. We can make a list of structural binding sites located in the V domain, which are composed in part of the CDRs and framework regions. That list, of course, includes catalytic sites and the protein A-, protein G-, and protein L-binding sites. It also includes the nucleotide-binding site. It would include the histidine-binding site previously mentioned. It includes a self-binding site. So we make a list of the binding sites and we find out the biological activity of these binding sites. Is that good enough to take you back to Paris?

Zouali: I'll do my best. So it's a class of antibodies that is infrequent and can be catalytic. Its biological activity is not mediated by the Fc part of the immunoglobulin.

Marchalonis: The other important point is that superantibodies have activities other than those predicted just from the classical CDRs. I think Don Capra gave a good example with his V_H434, which contains a framework 1 marker that allowed it to be a superantigen binder. In other words, the activity is not conditioned by the F_c, and the range of biological activities is more general than the binding specificity imparted by the classical CDRs.

Paul: Is there anyone who thinks that this term is unnecessary—that we could do without it?

Unidentified: I am not sure whether the term is appropriate, and I am not sure that all activities are not superantibodies.

Paul: You could quarrel with just the term "antibody" if you start with first principles. At this time we assume that all antibodies do not have the unconventional functions outside the CDR regions. If it turns out that all of them have the unconventional functions, then I agree that all antibodies should be called superantibodies.

Brahmachari: I think Sudhir defined it very well. There are many proteins with just one known function. We know now that there are proteins with multiple functions. So what we are trying to say is that antibody V domains in the past were thought to have only one function, but if they have additional functions we want to use the prefix "super."

Marchalonis: Groucho Marx used to say that he wouldn't want to belong to a club that would accept him as a member. But we know that all antibodies are not superantibodies. Even in the V_H3 family, only a small subclass will bind protein A or protein G. It depends upon a particular peptide sequence in the third framework. Some antibodies have extra properties and others do not.

Paul: And we know that some antibodies are noncatalytic. As our symposium is now drawing to a close, can I just take 30 seconds to say how much I am thankful personally to the speakers and to the participants. The last three days have been a high point. We will go back with lots of ideas. I'd like to thank our local hosts for your hospitality. This has been a wonderful academic experience and a wonderful personal experience.

Sarma: We have discussed the origin, evolution, and the mechanisms of antibodies, posing more questions as we answer the old questions. This Satellite Symposium has brought very distinguished scientists from all over the world to our country. Our young scientists have enjoyed excellent interactions with you. The support of Professor Sudhir Paul and Dr. Vijayalkshmi was very important in organizing this symposium. I hope you will go back with good memories and in the next symposium, will have answers to the questions raised here.

Vijayalakshmi: On behalf of everybody, let me thank the participants and our distinguished guests who agreed to our tough organizational conditions. On a personal note, I want to thank Sudhir and Sarma, who have worked with me on various organizational logistics, and Dr. Brahmachari for his hospitality.

Green: Can I say one word? This is such an enormously happy occasion for all of us who have been here and we enjoyed so much of your hospitality. There is something very somber I am thinking, though. Today years ago, Yitzhak Rabin was assassinated. I think this also relates to India, where Gandhi was murdered by a religious fanatic in the same way as Rabin. It is bad that we use the word "religious" for such abomination. In this gathering of international people who come together searching for truth and cooperation and friendship, it seems important to realize what terrible atrocities we can commit to one another. Yesterday when we came late to the symposium, Sudhir, Sasha, Sudhir's wife, and myself were actually discussing how in small ways evil can even pervade into supposedly objective scientific interactions. In Israel, this is a day everybody commemorates. I guess as scientists we have to not only be aware of the importance of objectivity and catalytic antibodies, but also how excessive fanaticism can be so disastrous. I apologize for taking time, but I felt compelled to commemorate this day.