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# General Discussion I

## Chaired by D. Capra

*Capra*: I would like to start by saying that I come here as a friend. My training is in Organic Chemistry. I took a Ph.D. in Physical Chemistry before I went to Medical School. I've never really worked on catalytic antibodies. I have been involved in molecular biology for a long time, and as the editor of *Molecular Immunology*, *The Immunologist*, and *Chemical Immunology*, I have certainly supported the work on catalytic antibodies. So, as a friend, I would like to challenge you in the following way. I'd like to ask where this field will be a decade from now. I've been around a long time, and I've seen a lot of fields become marginalized because nobody ever did the definitive experiments. Now, you can disagree with me, but it seems that nobody is doing the definitive experiments in this field. What everybody is doing is finding in antibodies, in a sense, a mimic of an enzymatic activity. It's not even a very good enzymatic activity most of the time. I would predict that if you took a thousand proteins off the shelf, and tested them all for whether they can cleave a peptide bond, you would find a few that could. There are only so many amino acids, and if you put them together randomly, eventually you come up with something that will cleave—a peptide bond. So let's accept that catalytic antibodies exist. The real question is: Are they important? Has anybody done an experiment to prove that these are important? Now, there are two ways they could be important. If we had a catalytic antibody that cleaved a nerve gas, and we could give it to people when they were going out onto the battlefield, or we could sprinkle it over a city, it would do something that we currently can't do. So one type of application of catalytic antibodies may be to dissolve garbage or clean up the environment. The other issue is: Are catalytic antibodies vital in biology? It's not hard to convince us that noncatalytic antibodies are vital to survival, because if you don't have antibodies, you get infections. If you give people γ-globulin, they don't get infections. If you don't have T cells, you get fungal infections. If you put back T cells with a bone-marrow transplant, the infections go away. So we need an experiment that will demonstrate that catalytic antibodies are vital in biology. Otherwise, what I would see for the field is that as more and more enzymologists are recruited, they will find an antibody (because there are millions of antibodies out there), which might do something that a particular enzymologist has been studying for years. And so I ask: If catalysis is what antibodies are about,

why did nature generate such a complicated way to make them? We have evolved thousands of enzymes in our body in very straightforward ways, and we know how to duplicate the locus, create a new specificity, and revise a specificity. Why does nature go through the trouble of doing two recombinations with a heavy and a light chain—putting two chains together—only to create a catalytic process for which enzymes already exist?

*Kohler*: I don't understand the question. What is the biological relevance of aspirin? Why has biology not come up with aspirin? Why are all of the pharmaceutical companies going for small synthetic compounds? The issue of biotechnological applications for catalytic antibodies is an important one, no matter whether they are vital in biology.

*Capra*: I agree. But can we survive without catalytic antibodies? I will accept that catalytic antibodies exist. No one doubts that, but are they important in human evolution?

*Tramontano*: They may or may not be vital in biology, but they may cause pathology.

*Capra*: Yes. To be important, either we need them to survive or they cause disease. Is there anybody here who thinks that catalysis is what antibodies are about?

*Green*: I think probably no one here on the chemical side thinks that, and no one here on the immunological side thinks that.

*Capra*: Is there someone who would defend the idea that catalysis is what antibodies are about?

*Paul*: I can defend the notion that catalysis is an important function of antibodies in disease and in the healthy individual.

*Thomas*: Your question about the importance of catalytic antibodies for biology is a very important one. You know that we are working on the anti-idiotypic approach in autoimmunity. Concerning possible biotechnological applications, you said that there are lots of enzymes with all the possible specificities and very high activity levels. This is not true. I worked for 30 years on enzyme technology. When there is a lot of enzyme activity, often the specificity is not the right one. In my opinion, the antiidiotypic approach is an incredible source of diversity. It is possible to get catalysts with new affinity, new specificity, and new activity. In nature, that is to say that with humans, plants, all existing life, you have enzymes of course, but all of the enzymatic genetic information is innate. In catalytic antibodies, some of the structural information is innate, but there is also a nice interaction with environment. Therefore, we accept gladly what evolution gives us, but we also try to introduce some new information that we can add to antibody structure. I think that in some ways the current status of catalytic antibodies is similar to that of enzymes at an early stage of evolution.

*Gabibov*: I liked the question about pathology because some answers may be forthcoming. After thinking for six years, I say catalytic antibodies may be the back side of evolution and it may be years before we define their importance. I like the model of the Bence Jones proteins because they might help answer the question of function. The rates of catalysis are very slow. But the quantity of Bence Jones proteins is so large that even slow catalysis can cause tissue damage.

*Capra*: I take issue with that. I think the experiments on Bence Jones proteins are interesting, but I wonder whether they have a real biologic significance. Of course they have significance in a patient with myeloma, but we are also talking about human evolution, and we are talking about how catalysis evolved in antibodies. Free light chains don't exist in normal people. I am not convinced that spending a lot of time studying the enzymatic activity of Bence Jones proteins is a reasonable ongoing avenue of investigation. Once you show that a Bence Jones protein has proteolytic activity, that's fine, but then if you want to deal at a larger level with an immune system that is selective and specific, you need to go back to the whole immunoglobulin molecule and show that the catalytic molecule plays a role in biology.

*Paul*: As we are dealing with several unknown variables in this discussion, the quality of the issues is progressively becoming diffuse and philosophical. The key practical question is: Are definitive experiments to determine the importance of catalytic antibodies being done? The survival of the field and further investments hinge on that question. Good scientists get into new fields only when they are excited by hard, experimental data.

*Capra*: The one advantage to being 61 is that I've watched a lot of different areas in biology and medicine become marginalized. You sit down as a group and say, what is the critical experiment we as a group most need to do? I can remember the early days of immunology and endless debates about germline vs somatic theory, until somebody stated and did the critical experiment. Once the experiment was done, the subject was closed.

*Paul*: I'd like to take a stab at defining an experiment that is straightforward enough to give us the straightforward conclusion that the catalytic function of antibodies brings to bear on biological systems something beyond the capability of the binding function.

*Paul*: Essentially, I will recapitulate a slide I presented. My goal was to measure the effect of antibodies on receptor binding of a neuropeptide, which is a physiological substance—VIP. Control receptor binding is measured in the absence of any antibody. VIP binding to the receptors in the lung is known to cause bronchodilation and an antiinflammatory effect. When I add an antiVIP catalytic light chain, the receptor binding is blocked completely. Another control is a mutant of this light chain that has exactly

the same binding affinity as the catalyst but has lost its catalytic function by a factor of about 100. Little or no inhibition of receptor binding is seen. So from this experiment, because receptor binding initiates the biological effect of VIP, I claim that the catalytic function is a more potent mechanism of blocking the biological actions of VIP than a noncatalytic binder.

*Kozyr*: In relation to the biological potency issue raised by Professor Paul, catalytic antibodies may be useful in the elimination of different viruses and antigens. In our lab we have a grant with our French collaborators to obtain catalytic antibodies to gp120 of HIV.

*Capra*: That falls into the biotechnology category, i.e., the use of a catalytic antibody as a drug. That would be great.

*Nevinski*: Concerning enzymes and specificity, we worked with the ribonucleolytic activity of antibodies from patients with autoimmune disease. The specificity depends upon the disease. For example, in multiple sclerosis, we see quite high specificity for RNA hydrolysis by antibodies, very unlike the specificity seen for conventional prokaryotic and eukaryotic enzymes. For example, the antibodies from multiple sclerosis displayed a specificity dependent on a single-base change in the RNA—a change to G.

*Capra*: Your point is that catalytic antibodies can have specificities that don't exist in nature.

*Nevinski*: Such monoclonal antibodies will be new instruments of investigation for basic science specificity studies. Often antibody-mediated catalysis is characterized by relatively low rates, but we have purified RNA-hydrolyzing antibodies from multiple sclerosis patients with specific activity as great as prokaryotic nucleases. Sometimes, it is clear that antibodies can be even better enzymes than conventional enzymes.

*Kohler*: I'd like to argue that catalytic antibodies cannot be vital in biology because they cannot be selected for by the immune system.

*Capra*: I've seen data at this meeting, e.g., the Buddy Green data—that of 100 hybridoma antibodies with binding activity for the hapten immunogen, 96 were catalytic. That stunned me because it implies that if you simply select for binding with the right ligand, then, in fact, selection for catalysis goes hand in hand.

*Tramontano*: In that experiment, 36% of the nonbinders were also catalytic. We have to ask, if you took a random array of antibodies, how many are catalytic—regardless of binding to the transition-state analog?

*Capra*: Yes, I was troubled by the catalytic activity of the nonbinders, but I was impressed that 96% of the binders were catalytic. Immunology is built on selection and specificity. If we don't have a way to select for catalytic antibodies, we cannot understand how they could have evolved.

*Paul*: We should not mix apples and oranges. When we speak of selection, the apple is the regular ground state of the antigen and the orange, the unstable transition state. Under the right circumstances, selection for catalysis could occur using either immunogen. In the case of the transition state, it may be too unstable to serve as an immunogen, and therefore analogs must be used to study catalyst selection. In the case of the regular antigen, rapid catalysis by the B-cell receptor (BCR) will deselect for catalysis only if the rate of catalysis and product release is greater than the rate of transmembrane signaling by the BCR necessary for clonal proliferation. Then, at a later stage in clonal selection, catalysis might be a favored event, because product release may permit B cells to escape downregulation due to high-affinity antigen binding and long residence times for the antigen on the B-cell surface.

*Neuberger*: If there is a normal function for catalytic antibodies in normal biology, tell me what it is. I will prepare for you a transgenic monoclonal mouse that has a single catalytic antibody specificity—the mouse should lack that essential function. Now, I need one more mouse as the critical test for a pathological function: give me a monoclonal antibody that occurs naturally, which you think causes disease. We will make a single amino-acid substitution that keeps the binding and removes the catalysis. We will then hope to show that the catalytic-model mouse has the disease and the binder mouse does not.

*Capra*: Let us apply a tough standard for catalytic antibodies. If you inject mice with a monoclonal antibody to an infectious agent, it protects you against infection. Is anybody proposing a straightforward experiment of that type?

*Paul*: Yes, we are attempting such in vivo studies using catalytic anti-VIP.

*Kohler*: Such experiments are also needed for biotechnological applications; for instance, clearance of drugs like cocaine. An antibody that only binds cocaine may not protect against cocaine overdose.

*Capra*: I am also looking for evolutionary selection pressures that force catalyst production. Sniffing cocaine probably wasn't it.

*Rose*: One possible experiment would be an experiment of nature. That is, to do Sudhir Paul's experiment by finding a patient who lacks the antibody and shows observable, presumably adverse, phenotypic effects caused by lack of catalysis—just as was done with thymus in DiGeorge syndrome.

*Marchalonis*: Actually, I wanted to bring up an experiment of nature as well. In humans and animals expressing the catalytic activity spontaneously, it must have been selected for or maintained by some mechanism. We know it is expressed in some V kappa regions, and not in others. Do you know of any diseases that are particularly associated with the catalytic sequences?

I'm just wondering if the answer to your question may already exist or can be found by sequence analysis in disease.

*Paul*: Unfortunately, linear sequence motifs associated with catalysis have not been described, so this type of correlation is difficult at present.

*Kohler*: I think it is helpful to look at the evolutionary framework for catalysis. The activity is mainly found in Bence Jones proteins and light chains. In other words, this is the first step the immune system takes to move catalysis from the innate to the adaptive immune system. Now, I think at this point the immune system may have decided it is too dangerous to go on to a full-length antibody with high catalytic activity. So perhaps nature did the experiment and then stopped further development of the experiment. This does not mean that Bence Jones proteins with catalytic activity are unimportant, because Bence Jones proteins accumulate in myeloma. But going on to artificially engineer full-length catalytic antibodies and use them for therapy may not be advisable. The catalytic activity is dangerous, and we may not be able to control all of the potential toxicity.

*Gololobov*: I'd like to propose a contrary view. Several catalytic autoantibodies specific for autoantigens have been described. So, presumably these antibodies can clear excess autoantigens. Perhaps the catalytic activity is a mechanism for maintaining tolerance.

*Capra*: Again, what are the experiments that you would like to see published, that you could go home and cite to your Dean or your department Chairman or present at a meeting, and say, "this experiment demonstrates that catalytic antibodies are crucial for survival." People who are in the cytokine business ask that question; if we don't have IL-4, we can't survive. People in the regular antibody field ask that question.

*Paul*: Here are two utopian experiments. First, knock out the critical genes involved in catalytic antibody synthesis and see if the catalytically deficient animals survive. Second, develop specific inhibitors of autoantibody catalysis that do not inhibit the binding of the antigen, and then see if administration of the inhibitors alleviates symptoms of autoimmune disease or induces dysfunction if the antibodies are physiological.

*Kohler*: Yes—designing a peptide or a small molecule that would bind and block the catalytic antibody would be useful. But the molecule should be nonhydrolyzable.

*Capra*: Let's turn to the question now of what sorts of imaginative things you could envision for a catalytic antibody in biotechnology—something that we cannot do today, e.g., split nerve gas or dissolve garbage or dissolve smog.

*Marchalonis*: What Sudhir put on the board is exactly the reason that we in the field would like to use catalytic antibodies in biotechnology. I don't think anybody can argue with the fact that a catalytic antibody is much

more effective at much lower doses than a normal antibody. Also, it doesn't form immune complexes and doesn't end up in the kidney. From that point of view, I think we all agree that even if catalytic antibodies are not vital in biology, they may be useful in medical science.

*Capra*: Perhaps the two aspects are interconnected. People in this room work on autoimmune disease, and they are trying to connect catalytic antibodies with destruction of thyroid glands or kidneys or other tissues. Also, catalytic antibodies might be naturally present in our bodies to rid us of HIV or pneumonococcus or fungal infections or kill cancers. Even if they are not vital to biology, they can be applied in immunotherapy of human disease.

*FitzGerald*: I would have thought that if catalytic antibodies were vital or could cure diseases, they would probably be more processive than the known catalysts. So if they were present with that level of activity, we would have identified and pulled them out by now.

*Capra*: So you are saying that if catalytic antibodies are really important in human biology, they would have been discovered a decade or two ago.

*FitzGerald*: The ones that have been discovered would have been better catalysts than they are.

*Paul*: Antibody catalysis has not been looked at directly until very recently. If we first screen for binding antibodies and only then for catalysis, we might be falling into a trap, and we might find only low-turnover antibodies. It is far better to select directly for catalysis from the autoimmune repertoire, as Al Tramontano and others are proposing.

*Tramontano*: Even low-turnover catalysis will be valuable when dealing with difficult reactions like peptide-bond cleavage.

*Thomas*: Even low-turnover catalysts are better than zero-turnover antibodies.

*Paul*: The physiologically relevant variable is the kinetic efficiency, not the catalytic turnover, i.e., when the ligand is present at nanomolar concentrations, as is usually the case in vivo. There is an unfortunate tendency in the literature to overemphasize turnover (or $k_{cat}$) values. It is the $k_{cat}/K_m$ value that determines the rate of the reaction. The antibody catalysts that are available currently display $k_{cat}/K_m$ values that are sometimes equivalent to highly evolved proteases.

*Paul*: I'd also like to comment on light chains in the physiological state. In fact, normal, healthy humans are reported to have about a couple of mg of free light chains per 100 mL of serum. The concentration increases, of course, in multiple myeloma. The second issue is that in the reducing environment intracellularly, light chains and heavy chains will exist in free state. You might well have catalysis of import by light chains intracellularly.

*Capra*: In B cells?

*Paul*: In B cells that synthesize the light chains, or in target cells that internalize antibodies.

*Tramontano*: If one could demonstrate that light chains are functional intracellularly, this will open the door to the possibility that light chains may process antigens within B cells for presentation to T cells.

*Capra*: So what I've heard then is that holding catalytic antibodies up to enzymes is not fair, because there may be reasons why we want slower catalysis than enzymes are able to afford, and catalytic antibodies may play a role in slower reactions. Second, I heard that catalytic antibodies have the capacity to respond to novel agents in the environment and create enzymatic activities that were not brought in by the germline. Third, I heard that some of the specificities expressed by catalytic antibodies are quite novel and different from the known enzymes. So these novel specificities may be of some evolutionary significance, and may be of vital importance to our survival. And even the activity of isolated light chains may be of importance, because inside B cells, light chains can participate in antigen presentation and can perform other immunological functions.

*Kohler*: Without having a disease caused by catalytic antibodies, you don't have the required proof.

*Capra*: Well, yes. But it is a young field. So, what kind of disease are we going to look for?

*Kaveri*: Asthma; lupus; thyroiditis.

*Capra*: I remain concerned. I'm afraid that five years from now a meeting like this will have more catalytic antibodies performing more functions rather than showing one catalytic antibody with a vital function in biology, or as a reagent that is useful in some profound way that we can't obtain otherwise.

*Schowen*: I recommend sticking to the vital role of catalytic antibodies in biology. There are people out there working as hard as they can to make catalytic antibodies capable of doing all kinds of technical things. This is probably not the best audience to solve that problem. Those people aren't worried about this audience, and the biological aspects probably are of greatest concern to this group.

*Paul*: Indeed, substantial dollars, manpower, and brainpower are being invested in transition-state analog development and reactive immunization. The expertise here is more of a biological nature. We are fortunately supported by chemists like yourself, Al Tramontano, Buddy Green, Tony Kirby, and others.

*Capra*: That's why I am focusing on the vital role of antibodies in biology. If you could get a catalytic antibody that cleaved HIV, it could be used in

a therapeutic fashion, although it is not vital in evolutionary biology. But I'm still looking for something to help me say that if I understood catalytic antibodies, I would have a better understanding of lupus or thyroiditis or some other disease. I'm looking for a vital link between catalytic antibodies and disease, or the absence of catalytic antibodies and disease. Either way will work.

*Rose*: I think the key word is "link," and I would like to use it in a different way. One of the elements that is lacking in autoimmune disease is a common link among all of the diverse autoimmune diseases. We know there are some factors that seem to predispose toward autoimmune disease—for example, female gender and HLA associations—but there's really nothing we can put our finger on. One slide that really impressed itself on my mind was shown by Dr. Kirby, in which he traced the efficiency of catalytic antibodies as opposed to enzymes. Most catalytic antibodies are at the bottom and the enzymes are at the top, with the exception of catalytic antibodies from autoimmune diseases, which are in the middle. If that's true, and if that is a common link among all of the autoimmune diseases, this would be a major step forward not only in our understanding in catalytic antibodies, but to me even more important in our understanding of a common element in all of the autoimmune diseases.

*Kirby*: I'd like to follow up that point and put in a word for the absent efficient catalytic antibodies from the designer approach. I don't think there is any doubt that if we can attain higher efficiency by design, we would expand the scope of what catalytic antibodies can do. There are clear targets—for example, we could tailor-make our own restriction endonucleases, or cleave peptides at a particular site of our own choice, and not the enzyme's choice, or activate a prodrug on demand at a particular site. But antibodies catalyze those reactions too slowly, even though enzymes can do the job with ease. The improvement of the antibodies, it seems to me, will be an incremental one. There is not any one spectacular experiment that we can expect. The autoimmune antibodies on the face of it are more efficient catalysts. If these are straightforward antibody molecules, we could learn from them the necessary structural pieces that we have to add to the designer approach. Then I think we have the springboard for really rapid advances. My own feeling is that this will be an incremental business. Learning about the autoimmune process will build up our general knowledge of catalysis in these systems, and provide a vital contribution which is already being made by this audience.