

Complement Factor H
29-Apr-06 - 1:00 pm - 4:30 pm

Greg Hageman: Thank you, Paul. It's a great privilege to be here. I appreciate the invitation from Emily. I don't see her. There she is. Thank you. What I'd like to accomplish today is, kind of, to tell you a little bit about what we do know about the role of the complement system in macular degeneration, kind of provide the basis that led us to the observation that factor H is involved directly, and then speculate a little bit with some relatively new data and talk about some things that we may or may not understand.

Before I move forward, I really would like to take this opportunity to recognize the wonderful group of people that I've had the good privilege to work with over many, many years, and particularly with respect to the talk that I'll give today, you know, Don Anderson, Linc Johnson, and Dean Bok, have worked with me for many, many years. Rob Mullins and Karen Gehrs and Steve Russell have been involved in a lot of the early studies looking at the role of complement system. More recently, Rando Allikmets and Mike Dean have been just a joy to work with, and I really appreciate the opportunity to have worked with them.

So, I think there is one observation, Al pointed it out very nicely, that has been the focus of our work for nearly 20 years, and that is that AMD is characterized by the deposition of drusen and some of these other abnormal extracellular deposits at very early stages of the disease, and it was really that single observation, being somewhat naive 20 years ago, that Link and Don and myself started asking the question, "could we use this observation that drusen are a key, are a hallmark risk factor associated with the disease? Could we use that to gain some insight into the biological pathways that might be manifest in the disease? The long, slow approach, but we really thought all along that, perhaps, this approach would be the most prudent one that we could take if, indeed, we would ever think about the idea of directing therapeutics towards early stages of the disease.

This slide, as I've said quite a few times recently, represents about 15 years of our work, but suffice it to say that at the end of the day, at least in our hands, that drusen were comprised primarily of proteins and other molecules that one could think of being associated with various immune-mediated processes. That's not to say that those are the only components that have been found in drusen, but by far and away the greatest composition of drusen do seem to be molecules associated with inflammation. And we focused, some years ago, we really started focusing on this complement pathway, I guess primarily because there were at least 20 different proteins associated with that pathway that we could identify in drusen. Perhaps even more importantly than that, we recognized years ago that the RPE primarily, and some of the choroidal cells, actually synthesize a great number of these molecules associated with the complement pathway.

Now the complement system, very briefly, is the principle effector in our host defense against, typically we think of it against microbial infection, but it really is a system, it's a very ornate system, that links the innate and acquired immunity systems together. And I won't go into the details, but the pathway is triggered by one of three sub-pathways, and all of those pathways feed through a common

enzymatic cascade that really affects the lysis of cells through the creation of this complex called the “membrane attack complex,” or C5b-9.

Interestingly, that’s a very important complex, but in many disease processes where this cascade runs amok, runs out of control, you end up with a great deal of tissue damage because of the creation of this membrane attack complex—tissue damage that you don’t want to see. And that really led us back to some of our early observations that, indeed, this MAC complex was very robustly present along the entire RPE choroid interface, and one of the first questions we really sought out to answer was were these increased levels of MAC specifically associated with macular degeneration.

And this is some work that Rob Mullins did as a graduate student in my laboratory, but just a huge amount of work. And Rob was able to show that, yes, indeed, there are much higher levels of membrane attack complex present, and primarily in the macula of individuals with AMD as compared with age-matched controls, and that was done in a set of human donor eyes that numbered somewhere close to 300.

We learned some other things along that way. We learned that, indeed, these membrane attach complex, although they are present in drusen, are also, in particularly in the macula, very robustly associated with the choriocapillaris. And, indeed, even in remnants of RPE cells that are being sloughed into drusen, you can identify co-localization between dying RPE cells and these MAC complexes. And Steve Fliesler helped us with a very nice set of experiments a couple of years ago where he actually was able to show that these MAC complexes were much more abundant in membrane fractions of eyes collected from donors with macular degeneration compared to age-matched controls.

So the stage was really set that it looked like we were dealing with the system complement cascade that was inappropriately controlled. One observation that bothered me a great deal for some time was this observation that factor H, which is an inhibitor of this alternative system, or the alternative pathway of the complement system, co-localized with these highly abundant MAC complexes. And that was counterintuitive to me; it seemed like if the system was being controlled by factor H, and there was a great deal of it present, that you wouldn’t see these terminal complement complexes. So that was kind of our first clue that perhaps factor H was not doing its job correctly. On top of that, of course, we knew from the studies that Margaret just pointed out, that factor H did lie within this RCA gene cluster, this cluster of complement genes, that lies on 1Q31. And it was really this observation, I think, that intrigued me more than anything else and really was that, kind of, final push that set us out to look at factor H as a candidate gene for the disease. And that was that these individuals with glomerulonephritis type II very often contain maculas that are just loaded with drusen and these drusen are indistinguishable, clinically, from drusen that we would see associated with macular degeneration.

I think, more importantly, we had three donors with this disease in or _____ and we were able to actually go into those eyes and look at these drusen from a compositional perspective; and, indeed, there was no difference between drusen we say in those donor eyes and those we saw associated with AMD.

That collective set of data, in addition to observations that MPGN type III families had linked to chromosome 1Q, and that a point mutation in factor H caused

MPGN type II in a pig model of the disease, really led us directly to screening factor H as a candidate gene. And I think the data have been nicely summarized by Margaret, but essentially what we were able to do were identify haplotypes within the factor H gene that were associated with increased risk or with protection, and Rando and Michael spent a great deal of time talking about that. Interestingly, we also did show that the MPGN type II was caused by the same risk haplotype as the AMD-associated haplotype.

What I'd like to do is, for the next few minutes, is really talk about where do we go now from a biological perspective. And I'd like to just kind of tell you what I think some of the key questions are. One of the first questions, of course, is, are there any data out there to suggest that Factor H, as a protein, really, or as a gene defect, even, is associated with the development of macular degeneration? And I like to show this example. This is some recent data where we've been able to show that a number of hemolytic uremic syndrome associated factor H mutations definitely cause AMD in a number of large families, suggesting that, indeed, mutations or point mutations in factor H can lead to macular degeneration.

There's been a lot of talk recently about what do all these variations in factor H, what do they have to do with the functional deficits of the protein? And I think that there's not a lot of new information along these lines at this point, but I'd like to give you some of my thoughts on that. I think we should be careful, now that we know that there are risk and protective haplotypes, or combinations of variants, out there. I think we have to be careful pointing to any single variant or restricting ourselves to studying a single variant in isolation without considering the combinations of variations that are present in any given protein. And I point out, you know, there's been a lot about the 402H variant, and I think we do, again, need to consider that in the context of the entire protein. And I think, you know, there are other variations that have been described in factor H at this point that could, indeed, alter expression, affect splicing, and, indeed, I think we're going to learn that this is a much more complex system than we first thought it might be.

Just a couple of observations from our group with respect to serum factor H, to try to bring that point home, is that if you look up here on a Western, up in the approximate 150 kilodalton range that factor H should migrate, and you really spread out proteins—you'll notice that these are individual patients—you'll notice there really is a lot of heterogeneity in the factor H immunoreactive bands in that region, and we really need to learn a lot more about how that relates to the specific genetic haplotypes. Interestingly, at least in a small subset of about 70 or 80 patients, we've actually shown that serum levels of factor H, when combined with the truncated isoform, are consistently lower in our AMD population as compared to the control population, irrespective of factor H haplotypes, so that's something we need to understand more about.

Interestingly, the 402 region, or the 402 variant, sits within a known region that binds C-reactive protein and acute phase protein that it's thought to target factor H to sites of tissue injury. We know that CRP is localized within drusen, kind of in a substructural distribution. And from some studies, Seddon, and Lipp, and others have shown that, indeed, there may be increased CRP levels associated with macular degeneration.

Recently we've been able to take this 402H version of the protein and show that it has a very much reduced binding capacity for CRP, so very preliminary data, but I think slowly we'll start beginning to understand more about the biology of these proteins.

Another major question in my mind is, you know, is there a trigger? What is the trigger for activation? Remember we've identified a gene that regulates the complement cascade, but, of course, the complement cascade has to be triggered in the first place; so there are any given number of molecules that are present along the RPE choroid interface that could be potential triggers for the disease and I think it'll take us some time to work through this. It's also possible that something else might be occurring, and I think we actually have to understand even more about the specifics of the alternative pathway that we're dealing with. There is this autoactivation of the pathway, which is called "tickover," that indeed could explain the etiology of the disease, just as could the so-called "amplification loop," which is probably the more common.

I think another very important question is, and some folks have hit on that this morning, is there a systemic involvement in this disease? Is this a macular-specific disease or is it a more global systemic disease? That's going to take some time to sort out, but I think there are indications from our group, and many other groups, that, indeed, there is systemic affect of the complement cascade, and certainly you can pull out some of these complement molecules as nice biomarkers for macular degeneration.

So let me summarize by, I hope I have convinced you that there is complement-mediated tissue damage at the level of the RPE choroid interface. It is more robust in the macula for reasons we don't understand. I think the alternative pathway of the system, in contrast to the classical or lectin pathway, is the primary pathway involved. That's verified somewhat by these observations that genetic variations in both factor H and factor B account for a significant portion of one's risk for developing the disease. I think we have a lot of work to do to understand the precise role of these isoforms, and various isoforms, in the etiology of the disease. But I do think it's time that we can start thinking seriously about assessing the complement cascade as a target for therapeutic treatment modalities.

And with that I'll stop. I would like to, again, acknowledge the hundreds and hundreds of people that have played a role in my life in collaborating with me over the last 20 years, and I'd really like to thank Rando for helping show me a new way in life. Thank you.

Questions?

Q: So, has anybody, or yourselves particularly, since you're sort of biochemically oriented, tried to reconstitute any of these events, say taking any of this debris, this mixed complement and factor H, etc., and sort of apply it to cells and see if you really elicit, and can one elicit any of these, like, lytic responses, or anything that would approach what you would anticipate?

A: Yea, you can certainly induce, you know, complement-mediated cell death, RPE cells in culture, so I know lots and lots of groups that are pushing along those lines, and I think we'll see a lot data coming out rather quickly.