

Complement Factor H

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Dean Bok: All right. Thank you very much, Paul. You've heard a lot today about cell biology, and genetics, and biochemistry, and I'll just briefly summarize for those of you whose plane might have been late, walked in the room late, missed a few points, I'll just hit on some of the salient features that were discussed today.

The early sightings regarding DNA complement pathway and immune system in AMD were made by Hageman, Johnson, Anderson, and Mullens some years ago, and there was skepticism about those results because the obvious criticism was that if you, indeed, find proteins associated with drusen, how do you know that they simply haven't adsorbed there nonspecifically; they are, after all, produced in great quantity by the liver. Factor H is one of the most abundant proteins in plasma, anywhere from 100 to 600 micrograms/milliliter, and so there was that caveat and that certainly was considered, but in 1999 they published a formal paper in which they proposed this hypothesis that the innate immune system might be involved in some way.

And this was given rather dramatic support by proteomics, for those who are skeptical of immunocytic chemistry by Crabb and his group in which they, again, observed the proteomic analysis of spots taken from 2D gels published in 2002, that, indeed, there were many components of the innate pathway in drusen. These drusen _____ hand-dissected and analyzed by proteomics.

So if you at the collection of proteins that Hageman, Johnson, Anderson, and collaborators listed, you'll notice that, indeed, there are these recurring sighting by immunocytochemistry of proteins involved in the innate pathway, and not only are the perpetrators, per se, present, but regulators, like vitronectin and clusterine, etc.; and activators, like beta-amyloid.

And so you can sort of think of a druse as a battlefield upon which the components on the innate immune system have gathered for battle and have left their broken bodies behind. There is also some evidence for the acquired immune system in the form of HLA, but that could very well be the residue of broken pigment epithelial cells that are incarcerated in drusen, as well as the processes from the dendritic cells, which are known to poke their little noses into the drusen; indeed, Hageman and colleagues have published a paper showing that a very high incidence of drusen have what appears to be a dendritic cell core in the center.

So, at the same time, the geneticists were working, and this is just to remind those of you who don't think much about immunity, like me, that we have an adaptive immune system, which involves B and T lymphocytes, and we have this wonderful innate immune system that we're born with that includes toll-like receptors, these are primitive immune molecules that go way back to drosophila, and then, of course, the complement itself, along with these other components.

The genetic studies started with genome-wide scans, and they go all the way back, as Margaret Pericak-Vance said, to 1998 and the genome-wide scans implicated a region on chromosome 1, ultimately involved in regulation of complement activation, so it's called RCA. And this is a slide that Rando Allikmets showed last year, I think, at this meeting, in which he showed the history of these genome-wide scans starting in 1998, and they're all sort of sniffing around this area, 1Q. Interestingly, you'll also notice that hemicentin is there. Hemicentin is also known as fibulin 6 that was reported by Schultz

and collaborators a few years ago, a very small group of AMD patients, in which they postulated that this gene might be involved in the etiology of AMD.

So that brought to, then, about 2004, and you see the names of lots of people who have been mentioned today in this room, to the observation that complement factor H might be involved. Complement is a collective term for about 30 different proteins secreted by the liver, but I'd like to point out that the retinal pigment epithelium, which we often think of as the liver of the eyeball, also makes these proteins, locally, and as the speaker just before me mentioned, there are three pathways for complement. One is called classic because it was discovered first, and it involves the binding of complement one to antibodies that are bound to the surface of the microbe that's being attacked. And there's something called the lectin pathway, which we won't talk about today. And, finally, the alternative pathway in the innate immune system and that involves a process whereby complement 3 is slowly lysed, slowly cleaved, in the serum and it's bound to the surfaces of cells, and upon activation, amplification of fragments of C3b are produced, and they can be produced by all three pathways. All three pathways converge on complement 3. Why is that important? It's because when they – upon converging on complement 3, we get this fragment of 3 produced, which then amplifies the binding to the cell surface. And whether you're dealing with the classical pathway or with the alternative pathway, you end up with so-called C3 convertase, _____ are different assemblies of units, but they all converge on C3 and attack it, and then you finally get these complexes down here, which involve another C3b addition on either side, whether you're dealing here with the alternative pathway or the classical pathway, and that produces the so-called C5 convertase; they then attack C5, C5 then recruits all of these other components of the membrane-attack complex. A hole is punched in the cell, the cell bleeds to death, and dies.

So that's why it was postulated that control of this pathway could be so important, and if something were to go wrong with control, the cell _____ actually inhibit complement activation, and it protects us, the host, from attack by the complement system and it exclusively regulates the alternative pathway of complement activation. It's primarily made in the liver for system utilization and that's very important; we don't want to mess around with that if we're doing therapeutics, but it can be synthesized locally by many tissues, including the retinal pigment epithelium. And I think in terms of local treatment, that's something that we should keep in mind as a possibility.

Now just to zoom in a little bit on factor B – again, the essence of learning is repetition, and this is a teaching session, so I'm repeating what was said in the talk just before me. Factor B is involved in this pathway and factor H is involved in this pathway over here. Now you heard about a paper by Allikmets, Gold, Hageman, and Dean involving factor B. We'll talk about this in just a moment. But factor H promotes the destruction of C3b. It binds to it, and in concert with Factor I, produces this fragment, IC3b, which is inactive, and so if C3b binds to our retinal pigment epithelium, factor H is supposed to jump in there and inactivate it, and if it doesn't do its job, our retinal pigment epithelium could die through collateral damage. In the meantime, the microbe is supposed to be attacked by C3b, and in concert with factor B, which you heard about. Now factor B is a promoter of the attack and if it works properly, you get this complex, Bb and C3b, and that, of course, is involved as the convertase. And there are all kinds of control points along here where one could potentially intervene in the processes, either trying to juice it up or slow it down.

So, since factor H, it was pointed out again today, that there have been other papers that have appeared. Michael Gorin's group here, implicating PLEKHA1 and also

LOC387715, which Margaret mentioned today. This one is sort of fading in terms of importance in the eyes at least of the geneticists, but this one has taken on some considerable significance. Dr. Pericak-Vance group has shown the powerful association with smoking, although this has not yet been identified as a true gene; indeed recently it disappeared from the website that I looked at as a real gene; could be an intervening sequence, we don't really know. Rivera, et al, amplified on this; this is Bernard Vaber's group, I believe, on this in a recent paper; then, of course, factor C2 and B, the recent Gold, Allikmets, Hageman, Dean paper.

So what is Factor B? It is a plasma protein, as I said, that activates the alternative pathway – activates, now, rather than protecting the host, it activates the pathway – and it's not unreasonable to think that could be involved in the etiology of AMD. Activation of the alternative pathway is initiated by cleavage of C3b bound to factor B, thus resulting in the formation of the C3bBb complex. And we already went over this, the role of Factor B and the activation process.

What is complement factor C2? That's been kind of put down a little bit today, but I don't _____ to include that in the scenario. It's a plasma protein that activates the classical pathway. It's not found in drusen by immunocytochemistry yet, whether it's there, I don't know. Activation of the classical pathway is initiated by cleavage of C4b bound with factor C2, resulting in the formation of this complex, which is the C3 convertase of the classical pathway. So it's not unreasonable to think that that could be involved, it's there for attack of microbes, and if microbes are not properly held under control, the consequences of that could be bad.

Interestingly, Rando mentioned this, and others mentioned it today, the B gene and the C2 gene are only 500 base pairs apart on the human chromosome, 6p21, and both of them are involved, in fact, in one of the haplotypes that they mentioned in their recent paper. Expression of these proteins, factor H, factor B, C2, they're all made by the retinal pigment epithelium, they're made in the choroid, they're made in the neural retina, so you wouldn't necessarily have to treat systemically to attenuate in some way the action of these proteins, and that's a hopeful sign, I think.

Other genes implicated in AMD to date – Rando wouldn't agree with this entire list, but I tried to be complete. I certainly included ABCA4, ApoE2 has been implicated— somebody said yes, another group said no, and Cathy Bowes-Rickman's group has recently published an interesting mouse transgenic in which, if you _____ ApoE4 transgenics high-fat diet, they develop all kinds of indications of age-related macular degeneration, like neovascularization and drusen, as so forth. Fibulin 5 is the one that Ed Stone's group published a while back; a very limited number of people. Fibulin 6 is the Dennis Schultz group; that's also called hemicentin. You've heard about factor H. Anand Swaroop's group published a toll-like receptor 4 last year. Mike Gorin, PLEKHA1. I didn't mention that if it is involved, that would include the acquired immune system, because this is thought to be an adaptor molecule, on the cytosolic surface of a white blood cell that is involved in activation of white blood cells in the acquired immune system. And then, of course, LOC387715, which we've already talked about.

So you've seen this summary before. I think the jury has agreed that inflammation could be a very important component of AMD and the associations are strongest in patients with early AMD and CNV, but weaker, at least from some of the papers I read, for geographic atrophy. And, certainly, the complement pathway could be considered a realistic target as long as one deals locally in the development of potential AMD treatment modalities. Thank you very much.

At this point, if there are any other questions for the speakers that you would like them to entertain, please stand up to the microphone and ask your question.

Dr. Swaroop?

Dr. Swaroop: So this question is either for you or Greg. So where do you think the complement proteins and, you know, other immune molecules in drusen are coming from? Do you think they're coming from RPE or they are coming from the other places? And also how come variations in CFH protein, like, say, Y402H or maybe other variants which may alter the expression. How come they are going to affect the accumulation of these proteins into drusen? I just want to hear your thoughts, I mean, if you have, sort of, wondered about, you know – How these molecules – where they are coming, and how they are coming, and...

Bok: Of course they could come from both the liver or they could come from the RPE or some other tissue that's making them. I think the important point is you may not have to tweak the system all that much in order to attenuate the process of pathogenesis. So, if you could have some local change in isoform, mediated by the retinal pigment epithelium, or the choroidal cells, perhaps you could do enough to slow the process down enough so that this wouldn't happen. In terms of, why do they collect there? I think of it, as I said, of simplistically perhaps, of a battlefield where the forces are not doing their jobs, so you call in reinforcements and more reinforcements, and they're all piling – all the factor H -- is piling up there. If you look at that beautiful druse that Greg showed, which is actually in the commentary in *PNAS*, as well, it's got the MAC attack right in the middle, and the entire thing is encrusted with factor H, and so if they're not doing their job, they might just be piling on and collecting there.

Q: (Inaudible).

Bok: Why does RPE make it? I didn't design the RPE. The RPE makes everything. You give me a molecule and I'll tell you if it doesn't make it?

Q: (Inaudible).

Bok: You think they have some completely different role?

Q: (Inaudible).

Bok: In other words, you think they have some completely different role? Maybe they do, but, for now, we don't have any reason to reject the fact that they're making it or that it's logical for them to make it. RPE makes transthyretin, for example. Why does it make transthyretin? It makes retinal binding protein. It secretes bacterially in the subretinal space. Why is it doing that? We don't have the answers to all these questions, but this cell, I have said in reviews, is, in my view, the most versatile cell in the body. It really is. And why it's doing all these things, I'm not sure, but it is a fact. It makes so much factor H, you can pick it up on a Western block readily, it makes that much. So you can take all of your patient's RPE cells and collect the isoform that they are making just from the conditioned medium. Greg, he asked you the question.

Greg Hageman: It makes a lot of sense to me that the RPE would continuously make factor H for its protective properties, right? To protect that basal surface, and then you end up with this highly risk haplotype that's just not doing the job. So that's another explanation.

Bok: Yes, did you have a question?

Q: I had a question for Michael Dean about the discussion of the evolutionary history of the CFH Y402H variant. At one point you said that it looked as though it had developed rather recently in response to a pathogen, and then you said that it was found in the African population, so I'm not quite clear on what your argument is there.

Michael Dean: Well what it looks like is that the risk allele is the most recent version of that protein, but it's not real recent; it happened quite some time ago. We see this with HLA, where there are many, many different forms of the gene which are all ancient and have been retained by lots of different populations, so it's not anything new to, you know, an immune-response molecule.

??-1: Actually, I have a mechanism for that. So in the last talk that was presented, it's been well known in the complement field for a long time that CFH, or certain pathogens recruit CFH to the cell surface and it's a way of generating an essentially immune evasion, and so the hypothesis – and this was the first time I'd really heard it as presented in your talk – would then be that the Y402H allele would then confer resistance to those pathogens and I think it's mechanistically consistent with the history of complement and what was shown in that last talk. I don't know if I made that clear, but one of the mechanisms for immune evasion is actually the use of CFH to trick the complement system into not attacking a pathogen.

??-2: That's a trick that microbes used to evade the attack.

??-1: Yes, so if in the ancient population you had to fight an infection, this would be a nice way to do it – the Y402H allele could confer resistance or, excuse me, prevent resistance to that infection and actually allow an attack. The experimental thing to do, then, is to take the CFH allele and show that does, in fact, not bind to those pathogens. I had a different question, though. Go ahead.

Q: I have a question for Dr. Bok and also Dr. Hageman, maybe. So this particular Y402H, I assume we inherit this thing ever since we were born, but why do we only get the disease when we reach old age, like 60 years old? Is there any reason...

Bok: Well you have to remember that it's a predisposing SNP, and, of course, don't forget the haplotype factor as well. It's predisposing – not everybody who has Y402H has AMD, so one answer to your question is that if you have a wonderful lifestyle and you walk, like Al Edwards said, to work, and you don't smoke – five times higher incidence in smokers, don't forget that, in some studies – and if you don't eat too many potato chips, you may not get it, ever – unless you live to be really old.

Al Edwards: May I make another comment.

Bok: Yes, please, Al.

Edwards: The other issue is that the disease begins to be diagnosed in that age range, of say 45s on, because, in part, limitations in technologies. So color fundus photography is not very sensitive. The contrast difference from a drusen and the, sort of, normal fundus background is not great, and you have to remember that the drusen are covered by the retinal pigment epithelium, as well. So if you use other technologies, such as the infrared imaging, you can see the disturbances at a lot earlier age. Now there's not a lot of work published on that; in fact, I'm not sure there's any, really. But we've done studies and presented them just in abstract form. And so the disease surely starts at least a decade or more – we know at least a decade before you can see it on examination or with fundus photography. And if you look at the original Beaver Dam classification paper that Ron Klein published, I think it was in the late 80's, maybe early

90's; he makes a comment in there that the earliest manifestation was a granularity of the retinal pigment epithelium, and that's something that you can quantitate, it's not something you can grade, it just an impression; and patients with that type of appearance, clinically, or on a fundus photograph, have large numbers of these reflectants and abnormalities that are characteristic of drusen with infrared images. So the disease starts earlier than we actually diagnose it.

Q: Michael, this is a little away from the theme, but at the end of the day I think if some insight about the role of chronic inflammatory mediators, particularly the role of homocysteine _____ with AMD. There have been some papers, but the link is very weak about the CRP levels in the people, and the homocysteine levels, and homocysteine has been proved as an independent risk factor for chronic disease, particularly cardiovascular diseases. Any feedback, particularly with respect to which _____ insight really in _____ part of the people and can trigger or something? Thank you.

A: Anyone want to take the answer to that one? Homocysteine and AMD? I guess not. Any other questions?

Q: A quick question. So complement factors – and I don't know who to address this to, so I think that anyone who spoke and knows a lot about complement in the eye: Systemically complement factors exist at a very high level. You spoke about that, so C5 protein is almost 500 nanomolar, C3 proteins are in the micromolar range systemically, so I'm really curious about complement factor levels in eye and any comment. Do they approach what we know to be true for the systemic system?

A: Within the eye itself. You mean in the vitreous or the subretinal space? I don't know if anyone's looked. Greg has looked at some AMD patients – looked at their serum, but I don't know...

Q: From a therapeutic point of view, it's going to be crucial, because to inhibit micromolar levels of an enzyme is very, very difficult systemically. And, so, if you've got very, very high concentrations in the eye, it's going to present a really interesting problems, and I think it's probably one of the first things we would want to get a handle on. So if you guys know the answer, that would be great.

A: I don't know the answer, but I will tell you that there have been publications doing 2D gel electrophoresis on both vitreous and aqueous in, sort of, control patient, controls beings like macular holes _____, so on surface retinal diseases, it would not be expected to disrupt the blood retinal barrier. And in those subjects you can see C3 easily detectable, these 2D gels; and subjects with diabetics, where the blood retinal barrier is disrupted, it is increased, so certainly there is C3 and complement factor H getting into the vitreous and the aqueous and this increases with breakdown of blood retinal barriers. So I think you could probably find the answer to your question in some of those papers.

Q: I have a quick question, and it's probably for Dr. Bok or Dr. Hageman, and my questions is, do we know when you have a MAC attack comp attack on the membrane of an RPE cell, is it going to die by apoptosis or by necrosis, or is it going to be a mixture?

Bok: As soon as you put that pore in the cell, there's a tremendous depolarization of the cell and loss of all the _____, so I don't know if it would have time to go through apoptosis. It's very fast, I think. It certainly is, you know, if we look at real time-lapse photography of cells that are being attacked, it's a pretty rapid process; they just foam up

and die right before your eyes. I haven't watched an RPE cells do that, but I have watched other cells. Yes, go ahead, please.

A: I don't know the answer to the question specifically, but there is some literature on the consequences of the initiation of the membrane attack complex on endothelial cells, and you don't necessarily go through a cell death. So, so the initial phases of the mac attack, the MAC deposition induce the nf kappa beta pathway, and there's a series of events, and I don't remember all of it – it's not really my area – but it is clear from these papers that you don't have to have cell death. The process can be aborted. And, in fact, you don't even have to form the pore to have internal pathways in endothelial cells being altered. I don't remember the references, but you can find them on the web.

MC (Paul): It's time to call this. If there are remaining questions, the speakers will be here at the front for a few minutes. We thank all of the speakers and ARVO for making this education symposium available. Thank you.