BBGuy Essentials 053CE:
"Alloimmunization is a BIG word!" with Chris Tormey
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Joe Chaffin: Hello, everyone, and welcome to Blood Bank Guy Essentials, the podcast designed to help you learn the essentials of transfusion medicine. My name is Joe Chaffin, and I'm very honored to still be your host. Today's episode is a discussion with Dr. Chris Tormey on why patients make those annoying antibodies against red blood cell antigens that can cause us so much trouble. I'm going to tell you more on Chris and today's topic in just a second…

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In the past week, I have heard from two different people that said they just don't understand why it takes "SO LONG" to train people in blood banking and transfusion medicine! They both said variants of, "I mean, it takes 5 minutes to learn ABO, and you're done, right?" (One of them MAY have been a surgeon, but I'll never disclose my source!). Well, obviously, if you are a regular listener of this podcast, you know things are not that simple! One of the complex things we deal with all the time is those antibodies that people form against the SEA of antigens on a red cell that are *not related* to ABO. I'm extremely honored to have Dr. Chris Tormey here today to guide us through those choppy waters.

Chris is an academic Clinical Pathologist and Transfusion Medicine specialist who is an Associate Professor at Yale University in New Haven, CT, where he directs the TM fellowship program. He has published extensively in many areas, including this one (so much that it's CRAZY! The dude is a machine!). He has been honored in many ways, including Young Investigator awards, and in 2014 was named to the prestigious "40 under Forty" honorees by the American Society for Clinical Pathology for outstanding achievement and leadership at a young age. Chris is great, and I think you'll learn a ton from him. So, here's my discussion on those red cell antibodies with Dr. Chris Tormey: "Alloimmunization is a BIG word!"

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Joe: Hey Chris! Welcome to the Blood Bank Guy Essentials Podcast, my man!

Chris: I'm very happy to be here, Joe. Thanks for having me!

Joe: It is my absolute pleasure to do so! I'm really excited about today's topic, because this is something that I started some of this conversation when I did an interview last year with Eric Gehrie about antibody formation in patients with sickle cell [NOTE: See BBGuy.org/030]. But this talk that you and I are going to go through today, I think is, a little more general. It's more generally applicable to non-sickle cell population and really the entire population, and I think it's really hugely important. I'm so looking forward to doing this. Chris, I just want to say right from the beginning, and this is what I'm going to title this podcast: "Alloimmunization, My Friend, is a BIG Word!" It's a big word, and I don't think people necessarily always understand it. So I'm going to just open the floor to you and have you help us out with just this big concept of alloimmunization. What does this big word mean and how is it relevant to us?

Chris: Absolutely, Joe. I'll try and sort of dissect this big buzz word in transfusion medicine. For those of you who are listening who don't deal with this on a daily basis necessarily, alloimmunization is really an immune response, not unlike that we see to vaccines, for instance. Fundamental rule of alloimmunization is that an individual lacks, in this case, a blood group antigen, usually it has to be an "immunogenic" blood group antigen, meaning it has to be capable of inducing an immune response. And most often, via transfusion or pregnancy, an individual sees that antigen and then develops a humoral antibody response against it. And then, over the course of about three to four weeks, you'll see an increase in antibody titers, and then that of course now puts that individual at risk, should they be exposed to that antigen again, for attacking the red cells that possess that antigen, or of course, in the setting of pregnancy, should a woman get pregnant and the fetus express that antigen on their red blood cells, those antibodies could cross the placenta and really cause some damage and hemolysis of the fetus. So alloimmunization is a big issue, and that's sort of the fundamental immunologic crux of it.

Joe: Gotcha. So, just out of curiosity, you know when we just look at red blood cells, Chris, they look like just nice simple little things. You know, they're these nice little biconcave discs that we've all seen in high school and college and, for some of us, in medical school. But things are kind of happenin' on the surface of a red cell, right? We're not just talking about a couple of different antigens on the surface of a red cell. It's kind of a "sea" of those suckers, isn't it?

Chris: Absolutely, yeah. Literally dozens and dozens of different antigens! Some that are protein-based, some that are sugar-based---they all play different functionality; in some cases, we don't even KNOW what the function is. We know that they exist, because people make antibodies against them in a setting of transfusion, but we're really only scratching the surface. And I think people in the general public think of the ABO and the Rh type, you know, "I'm A positive." "I'm O negative." They don't realize the complexity that blood banks deal with on a day-to-day basis in identifying the dozens and dozens of other antibody-antigen combinations that are potentially out there.

Joe: Well can you give us--just off the top of your head, just some of the buzz words, the names of some of these antigens that people should be aware of? What are some of the blood groups that are involved in this?

Chris: Definitely, yeah. So the ones that are of course most clinical significance to us, tend to be the ones that people know a little about to begin with. So, certainly ABO being probably "the king of all antigen systems" and the most important. We tend to--and whenever I give talks to my medical students and residents, people tend to think of Rh as, "I'm either Rh positive or Rh negative." But what a lot of individuals don't understand is, that refers to a single antigen in the Rh system, called “D.” And within that system, D is very important. But there are a number of others, like the “E [Big E]” antigen, the “e [little e]” antigen, the “C [big C]” antigen, the “c [little c]” antigen. Sounds like alphabet soup after a while!

Joe: Yes, it does!

Chris: Those would be definitely clinically relevant to transfusion and pregnancy. There's another big system called the "Kell system." When we tend to think of or ranking these, one of the ways I like to think about them is, if an individual gets exposed to the antigen, how likely are they to respond to it, make an antibody against it? After the RhD antigen, the one that we mentioned earlier that pretty much everyone knows about, the "K [Big K]" antigen in the Kell system is really important. It is highly immunogenic, and we see a lot of people making immune responses against that. And then we have others. Some of these were named after patients in which they were discovered (this is obviously before the HIPAA era). We have systems like the "Duffy" system, which have different antigens like Fy^a or Fy^b. We have the "Kidd" system, also Jk^a or Jk^b (these are abbreviations that we use in blood bank systems to abbreviate, and so we don't have to keep saying the names out again). And then also, the "Lewis" system, a Le^a and a Le^b. So, that would be some of the more clinically relevant groups that we deal with on a day-to-day basis.

Joe: So when we're talking about alloimmunization as we are today, Chris, are we primarily focusing on those groups that are *not* ABO, or is ABO included in this discussion?

Chris: Yeah, that's a great question. ABO is really an entity unto itself. It’s, I think, a mystery to many blood bankers, but we don't usually refer to ABO when we talk about alloimmunization, for reasons we don't still truly understand. As you well know, the ABO system, we start making antibodies against whatever we lack from very early in life. You don't have to be transfused, you don't have to be pregnant. So, alloimmunization in the context of what we're talking about today, Joe, mostly refers to the non-ABO antigens, the ones that essentially require having been previously transfused, been previously pregnant. So ABO is an animal unto itself.

Joe: Got it. Well, how often do we see this alloimmunization, Chris? Do we have data to suggest what proportion, say, for example, of general hospital populations have alloantibodies?

Chris: We absolutely do, Joe. We tend to think that when we look at essentially every person who has gotten testing in a blood bank, how many of them have an antibody (any of those classes we talk about, and some of the more even esoteric ones that we didn't mention)? People have found that for these “general patient groups,” maybe about 3 or 4% of individuals will have an antibody. But that number could be a little bit misleading, because it doesn't always include people who have been specifically transfused, so you're counting people who haven't gotten the exposure to the event. And when people wrote down and when they looked at---okay, again, as we said earlier, the transfusion is probably the biggest source of exposure to these antigens outside of pregnancy. And certainly for half the population, pregnancy obviously can't be a trigger for this. So when we look specifically at transfused individuals, and really drill down on that group, actually is a fairly significant number of those individuals. It's about 10 or 15% of those who are transfused develop an antibody. And the thing that we also know, is that when we look at groups of patients who are chronically dependent on transfusion, so, for instance, individuals with sickle cell disease, individuals with a disease called "thalassemia," sometimes, even individuals with malignant disorders like myelodysplastic syndrome, we see even higher rates, sometimes approaching up to 50% of transfused individuals. So really, depending on the population you look at, and the number of transfusions they receive, this really could be, or is a very prevalent problem in Transfusion Medicine.

Joe: Let's bottom line this. You're a clinician, you've ordered blood, and the blood bank calls you and says, "Your patient has an antibody," and they give a name that you've never heard or maybe you've heard, and you remember something about "Kell kills" or something like that. Something weird is happening. So what does that functionally mean to you? What are the potential consequences of someone having an alloantibody?

Chris: Yeah, I think the most striking one, and I think the most important one that we see upfront is, it's going to lead to complexities in transfusing that patient. So, one thing that we do at our hospitals, when we identify a new antibody, particularly on a patient who's in our emergency room, maybe in one of our ICUs, maybe on our medical floors, the first thing we do is call clinicians, just as you said, and let them know we have to identify the exact antibodies that are there. Once we do that, we then have to find blood that is compatible for that patient. And again, depending on the antibody, that can take minutes, that can take hours, sometimes that can take days or even weeks, if it's an extraordinarily rare antibody that the patient has formed. So I think that's probably the most pressing issue that comes up is, we need to find blood that's compatible for this patient that lacks the antigen to which they've formed the antibody.

What we also have come to find is that that's not the only complication. So, it's delaying care, undoubtedly. What some authors have actually found is that these delays in care, particularly in very vulnerable populations, could actually lead to increased morbidity and mortality. So for example, if you're a sickle cell patient who is dependent on transfusion, and I'm a blood bank telling you as a clinician, "It's going to take me hours or days to find compatible blood," we've definitely seen increased rates of complications for patients, because they literally cannot be transfused.

Of course, the other complication is that, should that individual inadvertently be exposed to that antigen on a subsequent transfusion, you're running the risk of red cell hemolysis, which is as a blood banker one of the most feared complications of transfusion that we worry about, and that can lead to all sorts of bad outcomes: Kidney damage, shock-like picture, and in some cases, depending on the severity of the antibody, and the immune reaction that it kicks off, in some cases even death. So, of course, that's the other major concern we always have, is that we really want to avoid ever providing incompatible units to patients down the road. But I think as we'll discuss a bit later in the talk, sometimes becomes very difficult for blood banks to continually identify these antibodies.

Joe: I literally just broke out in a cold sweat, Chris, when I you started talking about hemolysis! So, yeah, you're right: That's the blood bankers' nightmare! One last question about this, again to put this into proportion: Do we have data that lets us know...well, let me back up for a second. When we think about people having a bad outcome as a result of a transfusion, we usually think about the ABO mismatch, someone accidentally giving the wrong unit to the wrong patient, and the massive intravascular, immediate, acute hemolysis that that can kill people, of course. Do we have any data on how, what you just described, kind of that "non-ABO" type of hemolysis, how often that actually kills patients?

Chris: Yeah, we do, Joe. And I think we rely in these cases on these very large hemovigilance databases that we have here in the U.S., and the UK is another great example, in Britain, they keep very close tabs on this. I've looked at this data, and it's all readily available on the FDA's website [NOTE: Current link at time of episode release: https://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/TransfusionDonationFatalities/]. They sum, each year, transfusion-associated fatalities, and they break it down by ABO antibodies and non-ABO antibodies. And if you look at about 10 or 12 years worth of data on that website, you come to find that non-ABO associated fatalities in the US are usually either the second or third most common cause of transfusion-associated death in the US. So, it's a problem that is often not thought about a lot, but it is very prevalent and important. And if you look at the UK data, they find not as high fatality rate there, which is good, but what they actually find is significant morbidity. So, maybe about 60% of cases have moderate-to-severe morbidity, about 10 to 15% of cases have mild morbidity, where these patients have these antibodies. So, they are causing really significant problems, up to and including death for patients. And again, from a transfusion reaction standpoint, we tend to spend a lot of time focusing on pulmonary

reactions, so, people have respiratory distress when they're transfused. But non-ABO antibodies have sneakily become a very, very common cause of transfusion associated fatality in the US.

Joe: OK. Chris, thanks! I think that's a great overview and a great summary. I want to take a look specifically at a case that you've described previously, and just allow you to take a couple minutes to walk us through this case that was a real case that happened to you at your hospital in Connecticut. And let's learn from it. So I'll give the floor to you. Talk us through your case.

Chris: Sure, Joe. So the case started fairly innocuously. It was right around the holidays time, right around Christmas, I remember it very, very well. The patient was visiting his family from outside of our state. I'm based in Connecticut, for those who are listening, and he was visiting from northern New England. And while he was visiting his family, he developed a very significant GI bleed. He came to our hospital and required, essentially over a course of two days, what we would consider to be "massive transfusion" (more than 10 units of red blood cells), some plasma products, some platelet products as well. He was in a "step down" or ICU setting during that time, and family was not really coming to see him that closely because of that. As the issue started to cool down, the family now started coming to see him (again, this is holidays, right around Christmas), and one of his family members came to see us, actually came to see the nursing staff, and told the nursing staff, "We understand that our father has been transfused with a large amount of blood. Were you using the 'special blood products' that he normally gets when he gets transfused in Vermont where he's from?"

Joe: Oh, no!

Chris: Never a good thing to hear! I can tell you, Joe, there was nothing particularly special about the blood that we have given out at that point in time. So, I think very beautifully and fantastically in this case, the medical team contacted blood bank immediately, and said, "Were we giving the patient special blood products?" We said, "No." We still do an old fashioned crossmatch, an immediate spin crossmatch (if listeners don't know what that is, it's basically just a test to make sure the blood products are essentially ABO compatible). So we did an immediate spin crossmatch, it was fully compatible. We said, "Beyond that, there was nothing special about these." So we said, "Give us the name of the hospital where your dad had sought care previously, and we'll reach out to those folks and see what the story is." So when we called the other hospital...And if I back up a second, I'd say, all of the testing we had done for that patient was normal. So his ABO typing was normal. His Rh typing was normal. And there is a test, Joe, that we do called an "antibody screen," which is really the means by which we look for the presence of alloimmunization, the presence of antibodies in patient's plasma. The antibody screen was completely negative, which is usually what we get in cases, again, 95-97% of patients are going to have a negative antibody screen. When we called the hospital where he got most

his care in Vermont, we were a little bit floored to find out that he had had multiple alloantibodies on file there, including several clinically significant antibodies in the Kell system and in the Rh system. So, really quite problematic for us at that point in time, and it became a big shock to us that we had transfused this patient with essentially randomly selected units, essentially *massively* transfused the patient with randomly selected units, and now we were facing a real potential issue because he had had this history of these antibodies which we didn't have on file at our place and which we could not detect.

Joe: Oh boy. And Chris, can I interject? When he got these units, was there any issue? Did he have any reactions when he was transfused?

Chris: No Joe, he tolerated them very, very well! No suspicion from our standpoint that there was really anything wrong.

Joe: Right. Oh boy. So what happened next? I'm assuming when you heard about these antibodies, you sprung into action?

Chris: That's exactly right. So what we did was, we said to the team, "Now that we know about this history. Can you send us a new type and screen specimen on this patient?" And when we got the type and screen specimen back, lo and behold, we started now to detect the antibodies which were previously undetectable. This is within about three days of the last drop of blood being given to the patient.

Joe: Wow! That fast, okay.

Chris: Started seeing the antibodies reemerging at a fairly low titer in his blood. At this point in time though, he was actually doing quite well. Didn't have any signs or symptoms of any sort of transfusion reaction, but now these antibodies were starting to be re-detectable in his plasma.

Joe: Did you find anything new other than what they had said before?

Chris: We did, actually, so... [laughs]

Joe: OH NO!

Chris: Yeah. Pretty remarkable. In addition to the four antibodies that he had had on file at the other institution, we actually found a FIFTH antibody in a test called an "eluate" that we do. So, again, for those of you not familiar with the blood banking techniques, we can test for antibodies in an individual's plasma, but we can also see, are there antibodies bound to the transfused red blood cells that might not have yet made it into the plasma because of their titer? And, lo and behold, we found an antibody that no one had known about previously on this patient. So there was actually a fifth anybody present.

Joe: Perfect! (sigh)

Chris: Just what we needed actually. Yes.

Joe: Do we have a mechanism for you to check the units that you've transfused to see which antigens they carry?

Chris: Absolutely, we do. So most blood banks, I would say frankly, ALL blood banks, by standards and by regulations, have to maintain at least one segment from a transfused unit after they are transfused. So, in this circumstance, we had saved several segments from each of the units that we had issued, and we went back and tested all those, very, very acutely. And we found that every single unit that we transfused him was incompatible for at least one of the antigens.

Joe: Oh no! [laughs] You did not roll the lucky dice those days!

Chris: We did not! In Some of the cases, actually MOST of the cases, there were multiple antigens that he was incompatible for on what we had transfused him with. So it was a pretty scary situation to be in with a patient who, at that point in time, was having no symptoms or no issues at that point in time.

Joe: Well, so, everyone we're going to leave you hanging right there for a little while. We're going to come back to this case at the end of this discussion, and talk about how Chris managed this case, because it's a great story, and I'm excited for you to hear it. But, I want to just kind of jump back into the discussion about alloimmunization before we get to how you managed it and what you did. So let me ask you this: You've talked before, and you said it when we were talking about that case, that 95 or so percent, 95+% of people that you test in a blood bank don't have alloantibodies. Do we have---obviously that includes some people that have been transfused, not all, obviously, as you said---but clearly, there are some people that respond, and some people that don't seem to respond. Do we have any evidence or any theories as to why this is a problem for some people more than others?

Chris: Yeah, I think "it's a work in progress" is probably the best way to describe it. There are folks who work on this from a very "basic science" standpoint. So, there are some folks at my institution here at Yale, people like Jeanne Hendrickson and Stephanie Eisenbarth, and people around the country like Jim Zimring and Sean Stowell, who study this in mouse models. So they have amazing models where they can transfuse mice with humanized red blood cells and look at the immune response. And there are people like me who do research on the human side of the world to say, "Can we try and correlate some factors of transfusion, that surround transfusion, so peritransfusion factors?" And what we kind of

thought, Joe, and the way we kind of look at the world is, there are really essentially four points, four places where you can touch the patient, where it can impact alloimmunization:

- And one of those aspects is the **donors themselves**.
- Another aspect is, how do you **store** the red blood cells, and **what do you DO** to the red blood cells once they're out of the donor? Is there something happening to them while we store them in the blood bank in the refrigerator that could potentially influence the immune response to those individuals?
- We tend to think about the **antigens themselves**, as well. The antigens, of course, are a huge driver.
- And then, of course, probably the most important is, **what's going on in the patient**, the recipient? So is there a disease process going on? Are they receiving a medication? Are they super-inflamed? Are there things in the patient that could potentially influence the immune response?

And people are studying each aspect, so kind of think of it as four different train stations on the train track. People are studying each of those different stations to try and find clues that would allow us to say, "Okay, we can predict, or have a better sense, that this population or these group of individuals might be more likely to make an immune response than others."

Joe: Well, why don't we take just a few minutes, Chris, to go through those? I love the way you break that down into the four places where there's an opportunity for something to be a factor. And you mentioned them: The donor factors, what happens to the unit, the intrinsic red cell antigens, and the recipient factor. So let's **start with the donor factors** and just high level it for us. What's been done in terms of studying that, that would suggest that certain donor factors might be an issue causing immunization?

Chris: Yeah, I think that the most important thing that we've discovered in this domain is that, I think it is fairly logical, is that the degree of mismatch between your donor population and your recipients is going to correlate with the recipient's ability to make an [antibody]. So, one thing that's really important, again, sort of a high-level discussion point for people don't do this on a day-to-day basis, is that there's a term called "heterogeneity" out there. It refers to genetics, it refers to antigens, and what we find is that there's great heterogeneity, meaning there is a great variety of expression of these antigens in different populations. So, if you're someone of Northern Caucasian extraction, your red cell antigen profile may look very different than if you're of Mediterranean extraction, or if you're of Southeast Asian extraction. So, this great heterogeneity is actually what (in the U.S., we're such a big diverse melting pot) actually makes it somewhat problematic from the transfusion standpoint, because we have a lot of donors who may express antigens that our recipients lack. So, the most important finding we found is that **the higher the degree of heterogeneity between your donor and recipient population, the more likely you are to have an alloimmune response**.

Beyond that, there has not been a lot of other work done. I can say that Yale, here in Connecticut, is part of a big network that's actually studying a lot of aspects of transfusion, and we are actually working on a study right now trying to link donor factors, like donor diseases, donor age, to recipient outcomes. I think there's more to come in that domain, but we don't know a lot more about what donor factors may influence the immune response.

Joe: All right. Well that's fair. So, degree of donor-recipient antigen mismatch with the heterogeneity, as you said, but other stuff like age of the donor, gender, etc. we just don't know yet. Okay, that's fair. So, let's go to your second one that you mentioned, which is the **unit itself and how it's stored, how long it's been since it was collected, things like that**. What can you tell us about those factors?

Chris: Sure! Unfortunately, we have a little bit of conflicting data. So, some of the things that people looked at, and Joe, I'm sure you've addressed this in other areas, as well: Age of red cell units has been a very controversial area....

Joe: Oh, yeah!

Chris: And, of course, naturally people have looked at clinical outcomes, but people who do the kind of research I do have also said, "Well, I wonder if the **age of the units** could potentially influence the immune response? Maybe an older unit makes you more likely to respond to a red cell. Maybe a fresher unit is better." I think we have a number of studies that would suggest at the age of units doesn't make a whole lot of difference. And then, we have a couple of studies that suggest that, in fact, particularly in, for instance, a disease like sickle cell disease, that older units might actually be associated with higher alloimmunization rates. So, I think we're lacking that really big, definitive study. It's a little bit controversial.

The other thing that people have looked at is **modifications** that you make to the blood product. What some of your listeners may not know is that lots and lots of blood in the United States has the white cells removed from it, or "depleted." They're not entirely removed, but they are removed to about 99% of what you start with, usually by a filtration process. So people have thought, "Well, if you're giving an individual, a recipient, red blood cells with a big white cell contamination, maybe that's a trigger or a stimulus to the immune system." They've looked at, is there any influence of what we call "leukoreducing" units on alloimmunization outcomes. And again, a little bit of a controversy; some studies say that leukoreduction doesn't impact alloimmunization, and some people actually say that it actually prevents alloimmunization, or actually makes things a little bit better. So again, a little bit of conflicting data there.

One thing that we looked at, a lot of people may recognize that we irradiate blood products. It sounds really crazy what we do! People learning about this field, they go, "What are you doing to these blood products?" So we actually, to prevent a very, very bad disease called "transfusion-associated graft versus host disease," we actually expose red blood cells to a brief period of irradiation. It can be done by x-ray, it can be done by radioactive substances. And we've actually looked at, that process is known to somewhat damage red blood cells. We looked at irradiation here at Yale: We didn't find any association there. So, it seems that irradiation of products doesn't influence or make alloimmunization rates any higher.

So those are the main areas that have been looked at from the donor standpoint. I think, again, a lot more work needs to be done there...

Joe: All right. So, that's the discussion on the unit factors. Let's move to your third one, which, quite frankly, Chris, I'm hoping is going to be a little more exciting...[laughs]. No, I'm kidding. And this one, it actually makes sense that this could be more of an issue that we may have more data on it, and that's the antigens themselves.

Chris: Right.

Joe: And this is something I know that's near and dear to your heart, because when I look through your publications, I see this kind of stuff all over what you've looked at. So, you don't have 45 minutes to talk about this, Chris, but I'll give you the floor. What do we know about **the antigens themselves** and how they contribute to the likelihood that someone's going to make an alloantibody?

Chris: I'll try to get it done in no more than 44 minutes, Joe!

Joe: That works. No problem!

Chris: This is actually a very significant influence. So we had alluded to a little bit earlier in our discussion that you have to lack an antigen at baseline, and you have to be exposed to it. And that's really a fundamental crux of these making an antibody response. But what we clearly know is that some antigens are much more likely to lead to an immune response than others. And this term, this kind of ability to induce an immune response, also is referred to by another big word, which we call "immunogenicity." Immunogenicity can be actually calculated or tested, and some of the work we've done and some of the work others have done, has clearly shown that there is a rank order of immunogenicity. As we said a little bit earlier, I think, in the talk (or we alluded to), **the "king" of immunogenicity for the non-ABO antibodies is the D antigen** (the RhD antigen). We know that, generally, if you're a healthy individual or a relatively healthy individual, if you lack that D antigen, a very high percentage of the time, if you get exposed to it you're going to make an antibody response against it, some people think upwards of 80 or 85% of the time. If we

were giving sort of indiscriminately Rh positive or Rh negative red cells out in random, you'd have a huge population that makes these anti-Ds, and that's really problematic, particularly for women of childbearing age, because that antibody is going to really impact subsequent pregnancies.

And then, the next most immunogenic antigen (the next most potent antigen) is the "K (big K)" antigen from the Kell system. So, what we can actually say very definitively is, and this actually leads to some strategies that we might talk about a little bit later, is that if you can identify the antigens like the "big D's," the "big K's," the "Kidd A's/Jka's," that have very high immunogenicity, if your patient lacks those antigens, you might want to prophylactically, or from the get-go, give them red cells that lack those highly immunogenic antigens to prevent an immune response to begin with. So immunogenicity is a huge impact on the alloimmune response.

Joe: Forgive me, Chris, I can't let one thing that you said go. It's not really, I'm not challenging it, but I know you'll know where I'm going when I say this: You mentioned that in the studies with "HEALTHY people" that are D negative (RhD negative) receiving RhD positive blood, that you've got an 80-85% risk. But we have more recent data suggesting that that number is much lower in hospitalized patients, correct?

Chris: Absolutely. And I think that it bears more scrutiny, and that's why I tried to be very conservative when I gave that number out. Because, if you really look at the older studies that quote that 80% rate, these were truly healthy donors. They weren't getting transfusions, they were often injecting them with red blood cells. So it's not exactly the same. So yes, the rates are not nearly the 80% that we would normally see with a healthy donor population. I think people would still say D is still the most immunogenic antigen, but it may be that truly 80% rate, it may be actually significantly more like 30 to 40% range. Your hospitalized, possibly immunosuppressed, maybe even bleeding patients... people have looked in the setting of trauma, for instance, and found very low rates of D alloimmunization where routinely Rh-mismatched blood is given, sometimes less than 10% alloimmunization rates to D. So yeah, it's unfortunately not a "one size fits all" answer for that…

Joe: I understand. So, in some of the papers that you have published, including one back in 2009 that you did, and an update that you did in the British Journal of Hematology in 2016, you did some major math, my friend! You did. And I've got to tell you, you lost me at the first equal sign on these formulas! That's OK. I love math, but you lost me there. But, let's bottom line it for people: If you were going to name say the "top 10" most immunogenic antigens, as I think you did it in at least one of these papers, based on everything that we know now and based on some of the stuff that we'll talk about later with antigens fading and coming back, etc., could you give us that hit list? Could you give us that top 10 list?

Chris: Yeah, sure. So, I don't have it in front of me directly right now, but I can certainly say like the top five [laughs].

Joe: Okay, fair enough.

Chris: So D, we still think is the number one immunogenicity antigen. K ("Big K") is still number two. Jk^a (the Kidd A antigen) actually is much more immunogenic than we thought it was. That will get into one of the corrections that we made, Joe, was the fact that people were probably underestimating the number of Kidd antibodies out there, because that's one of those antibodies that disappears very, very rapidly and can reappear very quickly. Lu^a (Lutheran A), which is a little thought about anybody, but one that very, very commonly discussed for immunogenicity purposes. Again, it's an antigen that people don't see very frequently, but when they see it, they tend to make an immune response against it. And the E antigen; those tend to be like the big four or five that we worry about most. I worry about going further down the list also, because a lot of the data that we use there, these start to be very, very low incidence antibodies. So, you want... Your most robust calculations are generally going to be based on the antibodies we have most of. If you look in different areas, you might actually find different immunogenicity rates. And I'm familiar with a paper that was just published from India, that actually found very high immunogenicity for an antigen we don't really even see here much in the US, called the "MUT" and the "MUR" antigens, very high immunogenicity. So, one thing I would definitely encourage people who are listening--- if they're still listening at this point...

Joe: They're ALL listening! What are you talking about, Chris?!? [laughs]

Chris: ...that if you're doing this kind of work, or you're interested in this kind of work, I think there needs to be more studies done this domain, using diverse patient populations across the U.S.--- even across the globe. And I think we'll find that immunogenicity may be, in many respects, a function of what antigens are more common in your population.

Joe: Got it. OK. So before we leave antigen factors, and immunogenicity is huge, but I think we're also becoming more and more aware of another issue with antigens, and that's antigens that don't "look quite like they should." What can you tell us about **antigen variants**?

Chris: Yeah. So this was a really, I think important paper that was published by someone named Stella Chou at the Children's Hospital of Philadelphia a few years ago [NOTE: Chou ST et al. High prevalence of red cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. Blood 2013;122(6):1062-1071]. And what they did there, as we had already alluded to, Joe, was that they were prophylactically matching antigens for patients with sickle cell disease. And even when they were doing that, they were finding very high rates of alloimmunization, which is kind of paradoxical, because you would say, "We have specifically matched these antigens, we've gone to

great lengths to do so, and we're still seeing people make these antibodies." And what they found by doing a genetic analysis is that individuals, particularly of African-American extraction or African-American background, have a high rate of genetic diversity in the Rh gene. So, in the genes that give rise to those Rh antigens--a lot of diversity, which are these very, very subtle changes at the molecular level, which translate to subtle changes at the antigen level, which means even if you're using a serologically or an antibody-based match, there could still be some immunogenic change there that allows someone to have an antibody be induced. So, what this study has actually argued for is that in populations (again I think, we have to really study this on a population-by-population basis), in populations where there's potentially a lot of genetic diversity, you may actually have to go even further than just the serologic match, you may have do a molecular match to overcome some of these issues. And that is an important finding, but it's also one for the transfusion community that I'm not sure we're quite ready for, because there are not many places that are in a position to do molecular matching for swaths of their population. And also, it's important, I think, when we do take care of sickle cell patients, one thing to remember is that I think for many years (certainly when I was trained), we probably treated things as "autoantibodies" that were actually "alloantibodies" that were aimed against these genetic variants. So, certainly in a sickle cell population, you have to take these antibodies that we think are sometimes autoantibodies much more seriously.

Joe: Everything that you've just said is really a podcast episode in and of itself! There's a whole lot more we could do there, but we need to move on. So we've done, in terms of why people make these antibodies, we've talked about factors unique to the donor, unique to the unit (and the way the unit is stored and/or processed), and then we just finished intrinsic antigen factors. So let's talk about the last one, which is obviously hugely important, that's **what's going on with the patient**. What can you tell us about that? What things do we know and have been shown to influence that?

Chris: Yeah. So I think, again, I think we have much more solid answers here, and I think we have a couple of areas. So certainly, **disease status**: Disease status is highly important. There are certain diseases which we just know are going to require larger amounts of blood. We've already mentioned a bunch of those, like sickle cell disease, like thalassemia, and again some cancers where patients may not be completely immunosuppressed, like myelodysplastic syndrome. At my hospital, some patients, the only therapy for myelodysplastic syndrome they get is transfusion, so they're not getting other therapies. We do know that diseases that correlate with higher transfusion burden like those three absolutely will.

What we've come to find out also, and again this is a lot based on animal models, which have then subsequently influenced human studies, is that diseases that correlate with **chronic inflammation** also tend to lead to higher alloimmune responses. And I think people are unraveling the molecular and the basic mechanisms for this, but in general, things that induce inflammatory responses, like lupus, or rheumatoid arthritis, things of that

nature, inflammatory bowel diseases, they tend to ramp the immune system up and make the immune system just more cognizant of exposure to foreign antigens. So, we and a bunch of others have found very high rates of alloimmunization in patients with chronic inflammatory disorders. So those are disease states, I think, that have all been contributory.

We also think that **age** is a factor. So I think it's pretty clear that if you're very, very young (so under four months of age), or if you're on the older side of the spectrum, you're very unlikely to mount an alloimmune response (not impossible, but very, very unlikely to). And kind of the "sweet spot" starts to become when you're getting into teenage years up until about probably your 60's or 70's. So age is a huge influence. We've seen that in the vaccine world, as well, that some people require, as they are very young or very old, they require repeat exposures to a vaccine before they mount an immune response to it. Same thing with red cell transfusion. So, those tend to be some of the well-studied recipient factors, and certainly more work being done in this area, but those tend to be the big hits.

Joe: Is there data on women versus men? And can you separate the pregnancy issue?

Chris: It's really tough, Joe. I think it's very tough from a blood bank perspective to separate that out. Most of these studies that have been done are retrospective. So it's very hard to go back and say, "At the time that we detected this antibody in someone, say, a woman of childbearing age, do we know whether she had had a transfusion exposure or maybe she had a pregnancy exposure?" My feeling is, I think there's a little bit of data out there, I think the "female issue," if you are a woman, you probably are, if you even correct for pregnancy, there are some studies that would suggest that women maybe are SLIGHTLY more predisposed to alloimmunization. When I was coming up in medical school, residency, and in fellowship, it was always thought, "Women form alloantibodies at rates of 2:1 or 3:1 to men," that's a pregnancy influence. I think the relative risk is probably shifted slightly more to women, but I don't think it's as exaggerated once you correct for pregnancy. To that point, a lot of the studies I've done have been at a VA hospital, where, honestly, 95% or more of our patients when we did these studies were men and they had very high alloimmunization rates. I think that we cannot discount it, but I think it's probably a very minor contributory factor.

Joe: Thank you for going there with me. And everyone listening, Chris has published multiple things on this. One of the things I would refer you to (and you will find this on the show page for this episode at BBGuy.org/053. Just look for this episode there), Chris and Eric Gehrie published an article in (I want to make sure I get the reference right) "Transfusion Medicine and Hemotherapy" in 2014, talking about a lot of these factors that we're mentioning, and they get into a lot of stuff that's deeper than we've been able to go, including some stuff that suggests that you may only be able to respond and make certain antibodies if you have a certain HLA type [NOTE: Gehrie EA and Tormey CA. The Influence of Clinical and Biological Factors on Transfusion-Associated Non-ABO Antigen

Alloimmunization: Responders, Hyper-Responders, and Non-Responders. Transf Med Hemother 2014;41:420-429]. And again, we don't have time to go into that. But, please check out that article, because there's a lot of great stuff in there.

Okay, so Chris, I think that's been a great look at some of the evidence for what we now know about why some people respond and why some people don't. Obviously, there's a lot of good stuff in there. There's a lot of questions still remaining, but I'd love to get back to your case, and just for those of you that are hanging with us, Chris described the case of a 77-year-old man who had a completely negative antibody screen, got multiple units transfused. Turned out, he actually had a history of four different antibodies, and they found even a fifth one after they transfused him. So, Chris, let's talk about the things that relate to that case. Let me ask you a question, because I know that clinicians would want to know this. So let me ask you a straightforward question, and you be honest with me. Did anyone in your transfusion service do anything wrong in this case?

Chris: No. Yes, I would say, honestly, nothing was done wrong here. Nothing really done outside of what we normally would do, which is to get a specimen, to do complete testing on it (do accurate testing on it), find negative results, and then provide blood that was compatible by immediate-spin crossmatch. So, yeah, unfortunately I think we were victims of immunology and physiology in this case.

Joe: And so let's explore some of that and why these things happen. The first thing that I think everyone should notice is: This guy had antibodies, and when you tested him, they were gone. And we do a very fancy name for that, which always makes me think of an early 2000s rock band, which is "**evanescence**." So what does that mean and is that common, what happened with this guy?

Chris: Yes, it is. So, when we were doing some of these initial studies, I remember very well, I was actually a resident when I started doing some of these studies, and I sort of was going through... We have this very old-fashioned card system that we keep in parallel with our computer system. And it really gives us patient's testing data from their first time they were tested right through the most present specimen. And as I was going through logging patients who had antibodies, it became very apparent to me that with patients getting sequential testing, testing that was positive to start with when we first identified the antibody was not staying positive. And I brought this to the attention of the person I was doing the research with, a guy named Gary Stack at Yale, and we looked into this, and we said, "There's not really a good name for this phenomenon!" People had referred to it as "evanescence" in other aspects of vaccine literature, so we said, "Well, maybe we'll apply this name to Transfusion Medicine as well." And to more specifically answer your question, yeah, this is now I think a very well-characterized phenomenon, where antibodies will disappear from detection over time. Now are they truly GONE? It's hard to say. I think what we can say for sure is that the antibodies...and for those of you who don't work in this field, antibodies, we often measure them by "titer," so how strong they react. So when you hear

me refer to this word as titer, it's really just, what is the strength of the antibody? What we know is that these antibodies (and Joe, I think our estimates from the studies that we've done show that about 2/3 of antibodies that have developed can become undetectable over time), we know that they drop below the level detection of our assays and the testing that we do.

Joe: That's terrifying! I'm sorry, Chris, I just have to stop you there because that's terrifying!

Chris: Right, and it's a real challenge for the blood bank community, because it's a process that not only occurs in a large swath of patients, but the timeframes to loss of antibodies can be shockingly fast. So what we looked at, again, because we had this really nice database of being able to look at patients sequentially over time, is that sometimes these antibodies became undetectable in a matter of weeks after they were initially developed. So if you're not doing really fast acute testing, it could be that someone is transfused on January 1st, and they come back your hospital on June 1st, and they may have made an antibody which you can now not any longer detect. So that's the phenomenon of evanescence that you were referring to earlier.

Joe: So, let's talk about another thing that happened, and that's that this particular gentleman had care at another hospital, and transfusion at another hospital, and then came to yours. And I think you call that "**record fragmentation**." Again, is that common?

Chris: It is, Joe. And again, some of the studies that we've done, and then some of the studies that have been done by folks like Mark Yazer and Meghan Delaney have shown that... And, I think it's kind of, everyone knows this and you just kinda have to quantitate it and put a name to it for people to recognize it. So, what we did was, we did a study, because I work at both Yale New Haven Hospital and the VA in Connecticut (which is pretty much down the road from Yale), we looked at the number of patients that just went back and forth between those two hospitals, and we found that about a quarter of them went back and forth between the two hospitals, and had some aspect of transfusion-related care. They got a transfusion, got a type and screen. And, kind of shockingly, and again, disturbingly to us, that antibodies that were on file at our hospital (or vice versa, were on file at Yale and not on file at the VA), about two-thirds of the time, there was a discrepancy in the records. Meaning, patients were going back and forth, and there wasn't good communication between the two hospitals that were close by. And, because of this problem of antibodies disappearing from detection, something would be detected at Yale, and not detected at our VA, or detected at our VA, and then not detected at Yale. So, a very scary situation. And actually, Mark Yazer, I mentioned earlier, and Meghan Delaney, they looked at this for patients with sickle cell disease, and they found that large numbers of patients with sickle cell disease who have antibodies will visit up to three or four hospitals over the course of, say, a year or two period, or a three year period that they did study. So, this is a phenomenon that happens with general patients, it happens with

patients with sickle cell disease. Probably, for a lot of our listeners, they don't always seek care at only one place. But the problem is, we don't have robust ways of communicating this information from hospital-to-hospital where a patient may seek care.

Joe: You're absolutely right about that. And again, maybe we'll talk about a little bit more on that in just a second, but let's go to the third thing that happened in your case, and that's, it was almost an aside, but you made an important point about the fact that when you went to test this patient after he had been challenged with 12 units of ultimately proven to be incompatible red cells (appeared compatible at the time) that a **NEW antibody** was found that was not previously noted in his previous care, an anti-S that was seen coating the red cells in the eluate, as you put it. So, again, let's just hit this at the bottom line: Is THAT something that's common, where people have a particular antibody profile, but, "OOPS, there's one more!", or something along those lines?

Chris: Yeah, absolutely, Joe. And again, I think, unfortunately for the blood bank community, it is pretty common. This case really kicked off a number of investigations that we did to really look at, how is this a problem? So, even if a patient receives care, let's say, theoretically, only at your hospital, you really do have to do follow-up testing of these patients when they get transfused. And again, if you wait too long to do that follow-up testing, or if you NEVER do a follow-up test on that patient, you're never really going to be able to detect those additional antibodies. So we call this phenomenon "**missed alloimmunization**," and we began doing some kind of fancy mathematical hoops, and jumping through different hoops. We calculated that, based on the number of patients at our facility who got a transfusion that either never got a follow-up test, or who got a follow-up test sometimes maybe a DECADE after they were transfused, the likelihood of us detecting an alloantibody is probably only about 30%. So, that's really scary! That's REALLY scary that only a third of the time is our follow-up testing really effective. And I think what it really comes down to, Joe, is that we don't have a surveillance system in place for detecting newly-developed antibodies. We kind of rely on the fact that patients are going to get type and screened immediately before transfusion. That episode could be completely resolved, and they may not need another transfusion for a month, a year, ten years, or maybe up to 15 years. And we don't then have a system in place after the transfusion to actively surveil for these antibodies. And, when you mix the factor that lots of these antibodies disappear from detection over time, that lack of a rigorous structure of follow-up testing after transfusion can wreak havoc on your ability to actually identify additional antibodies.

Joe: Let me just throw it out to you: You've talked about some significant problems with evanescence, with record fragmentation, and with missed alloimmunization; just thumbnail it for us. And again, we can't solve all the world's problems in the next few minutes, but what are some things that maybe people should think about that might help get past some of these challenges?

Chris: Yeah, I think the biggest stride we make against this is, try to increase the information, try to increase the communication of the information. Simple things like giving a patient a wallet card, or an identification card that lets them know they have these antibodies. That at least gets them involved in the process. We've never really done this study, I think would be interesting to know, but how many patients who have an alloantibody actually even KNOW they have an alloantibody? Patients normally know their medical history, they know their medical allergies; I'm guessing most patients don't even know this. So, empowering the patients, giving them information about this...In many cases, in the case that we had talked about earlier, we were lucky that the patient's family let us know that this patient had an issue, that we were actually able to recognize it earlier, rather than having it become a disaster. So, increased portability of information is critical.

One thing that I think I've advocated for, I think we're seeing increasingly advocated for in the U.S., and it actually exists in other regions (it exists in Canada, it exists in France, exists in the U.K.) is some sort of anybody database or registry, whether that's a regional database, whether it's a national database. Something where you as a hospital could look and inquire about a patient's history. As you know, Joe, it's very difficult, and I'm sure people listening know, it's really not practical for us to call and get a history of transfusion or pregnancy for every single patient who gets type and screened. We're doing thousands and thousands of these tests a year. It's just not feasible. If you had an easily accessed database, though, where we could, you know, a few clicks or a few typed keys, look up a patient's alloimmunization history, I think that would make the communication of antibody history much more feasible. And I think in many respects, that's why we've seen lower rates of these types of problems in countries like Canada and the U.K. where there are these either regional or national databases.

Joe: I completely agree with that, and it's something that I've been... I'm an older guy than you are, so I've been around a little bit longer, so that just means I've had a chance to be frustrated by that fact a little longer than you have [laughs]. It is frustrating! So, that is an absolutely great point. Other things that, again, if we had more time we could talk more about include (and you mentioned some of these), increasing our genotypic matching between donors and recipients, certainly, you talked about detecting antibodies, testing the patients with follow-up. And one simple thing, Chris, I'll let you expand on this, one of the best ways to stop alloimmunization is to make sure that our red cell transfusions are appropriate in the first place, right?

Chris: Preventing the alloimmunization event to begin with by not giving unnecessary transfusions is a huge way of making this work much better. So, adhering to judicious transfusion criteria. Again, when I was a medical student, not that long ago, I was told by some people when I did my medicine rotations, "If you only have to give one red cell, don't give the transfusion. You're supposed to give two or more." We've now realized that that's not really the way you're supposed to practice transfusion. You're supposed to give judicious transfusions. Read local guidelines. Consider what is the benefit of the

transfusion for your patient? Yeah, the more you can limit exposures, the better I think for patients.

Joe: Completely agree. OK, so we've kept people in suspense long enough, Chris, we've got to go back to your case. So, I'll set the stage and give it back to you. So you've got a patient that you've fired 12 stinking units of red cells into that looked compatible. He's responded OK, doesn't seem to be having any problems. But now, you have information clearly showing that he has the potential to have a problem. He's got 12 units of incompatible red cells running around in his blood. He hasn't, and correct me if I'm wrong, but he hasn't had any kind of a reaction yet, but it's like a time bomb. So what did you guys decide to do?

Chris: Yeah, Joe, so you're 100% correct. So he was a ticking time bomb. In this case, we actually sort of calculated, sort of "back of the envelope" calculation, that the vast majority of his circulating red cell mass at that point was incompatible red cells. So, we were on the cusp of what we call a "delayed hemolytic transfusion reaction," where, as we said earlier, the antibodies were coming up after a few days of the exposure, we were getting this history. Kind on a predicted basis that you could predict that the next thing that was going to happen was that as these antibodies ramped up in titer, and most of his circulation being incompatible blood, he could have a very severe hemolytic transfusion reaction. There are a couple options in that case. Some people would do "watchful waiting." That really didn't seem like a great option. This was an older gentleman, he had some comorbidities; he was a real survivor, but he had heart disease, he had multiple malignancies. Like I said, he was older. He was not going to tolerate watchful waiting. So, what we actually ended up doing in this case was a prophylactic red blood cell exchange, where we actually knew that the reaction was coming, we could see it like storm clouds on the horizon [laughs] we could see it coming. What we did was, we intervened, we exchanged him with about 7 or 8 antigen-negative, compatible units that lacked the antigens to which he had made a response. It took us a while to compile those because of the number of anybodies he had. But we did a late night procedure. He tolerated it very, very well. And we essentially evaded having him have a severe hemolytic transfusion reaction.

Joe: That is awesome. So, he survived and was able to leave the hospital?

Chris: He was! He did quite well. We had stockpiled units for him because, believe it or not, Joe, I don't think we got into this in the actual details of the case report, but then a few days after we did the exchange, the GI bleed opened back up again.

Joe: Oh no!

Chris: He needed another 4 or 5 units of red cells, but at that point, we had stockpiled antigen-negative red cells. Our blood donor center was so helpful, the Red Cross, in

getting us a large swath of these red cells, not only for the exchange, but then also for subsequent GI bleed. We got him stabilized, we sent him back up to Vermont. We communicated to his hospital back in Vermont the additional antibody that we had identified...

Joe: Good call!

Chris: ...should he need additional transfusions there, it would be important that they know that he had this initial antibody. Because one thing that we do know is that these antibodies, once they come back, they don't always stick around, that they go away a second time. So we wanted to communicate about this additional antibody that we detected.

Joe: That is fantastic. Well, Chris, this has just been an awesome discussion. I am so happy with the things that you were able to point out, and I think this is going to be really, really useful to my audience. So, I guess I'll just leave the floor to you for just a sec. Is there anything else that we should be aware of with this? Anything else that we didn't talk about that we should have talked about?

Chris: No, I think we covered all the important bases for sort of like a "primer course" in alloimmunization, and I hope people found it interesting, and hopefully inspire a few people to maybe want to study this themselves.

Joe: Awesome! Thank you so much, my friend. I really appreciate it.

Chris: Thanks, Joe.

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Joe: Thank you all so much for listening. Don't forget, you can find references and more cool stuff at the show page for this episode at BBGuy.org/053. Also, please take a minute to rate the podcast on Apple Podcasts, so more people can learn about the essentials.

I have a series of fun stuff coming in the next few months! The next episode is one I did in response to an avalanche of requests. Yes, I'm finally going to cover ABO discrepancies on the podcast! That's coming in a couple of weeks.

But until then, as always, I hope that you smile, and have fun, and above all, never, EVER stop learning! Thanks a lot. Catch you next time on the podcast!