

## Nanotechnology Symposium 1-May-06 - 8:30 am - 10:15 am

**Ritch:** Paper by Ellis Bankey. OK. We are a minute ahead of schedule. Dr. Zhao from Sichuan University in Chengdu could not make it to ARVO. He was going to talk about energy from nanodevices and molecular self-assembly. So I guess we'll go ahead with Dr. Sunil Shaunak and he's from Imperial College in London and he's going to talk about the therapeutic potential of dendrimers in disease of the eye.

**Shaunak:** Thank you Bob, I'd like to thank the organizers for the invitation to speak at the meeting today. I'd like to start by introducing two concepts from different fields, which we've married together to try and address the problem of scar tissue formation after surgery. And the first is about dendrimers. And dendrimers are large carbohydrate based structures that are made in generations as you've just seen on the screen. Now cationic dendrimers and the ability to link molecules to the surface cationic dendrimers has been established for some time, but the problem with these molecules is that they're actually toxic when you give them to animals. And so about five years ago we with some NIH money got involved in using anionic dendrimers and linking molecules to their surface because the beauty of anionic carbohydrate structures is that they are not toxic in even very large doses when given to animals. So that's the first problem from chemistry that we solved.

And the second concept that we wanted to address was that we became concerned that we've all been working with small molecule monovalent medicines for a long time and perhaps the time of these was over and what we had to do was to rethink the whole idea of medicines and start to think about polyvalent medicines, larger molecules where you have to have several ligands binding to several receptors in order to get fundamentally new biological responses.

Why do I say this? Well, here's an illustration of what we're learning from the new immunology. We know that surgical injury triggers the formation of ligands, small molecule ligands, which have to be stabilized on cell surface heparin proteoglycans and reach a certain concentration before they effectively trigger the receptor for which they are required. The best example of this is chemokines.

The biological experiments that have led to these sorts of concepts really come from this kind of slide. We've known about the inflammatory response that is triggered by bacterial lipopolysaccharide for a long time. More recently, we've understood that this is actually all mediated through toll-like receptor 4 on the cell surface. But what's been very exciting is that surgical injury causes the release of tissue enzymes, which cause the release of small molecular weight molecules of heparin sulfate, which also trigger toll-like receptor 4. So this is almost certainly why when we see patients who seem to have a septic shock type syndrome after surgery and we can't find any bacteria and they don't respond to antibiotics, it seems to be somewhat of a similarity. It is because you've actually got two ligands: bacterial lipopolysaccharide, and small soluble low molecular weight heparin sulfates which are triggering toll-like receptor 4 causing the receptors to aggregate and the polyvalent mechanism triggering chemokines, cytokines, and the inflammatory response.

Well in order to try and bring chemistry and immunology together we had to identify a meaningful biological or clinical problem that we wanted to address and we met up with

Pankow in London and realized that the problem of scar tissue formation after the coma surgery was a very real one. And it gave us something very real and tangible to address as an endpoint. And if you stop and think about surgical scar tissue formation, you can think of it as injury causing dendritic cell and macrophage recruitment at the same time you have profound angiogenesis that's associated with it, which causes even more immunological cells to come along, at least the T cell infiltration, chemokines, cytokines, inflammatory responses, and eventually scarring. And, really, as physicians we've been lagging a long way behind in this field because what we tend to do is put boulders at the bottom of the waterfall rather than at the top of the waterfall, be they steroids or anti-TNF antibodies.

So the first thing we wanted to do was to try and interfere with the upper arm of that cascade. And what we argued was that we should be able to make an antagonist, a dendrimer laced polyvalent antagonist, which would interfere with the ability of soluble low molecular heparin sulfate to bind to toll-like receptor 4, and in fact we did do that. We made a molecule called dendrimer glucosamine and in one of the experiments here, which I am showing, what we've done is take human monocyte-derived macrophages and pre-incubate them with the dendrimer glucosamine and then add lipopolysaccharide.

And what you'll see is that the tall bars are the positive controls for chemokines, \_\_\_\_\_ alpha, \_\_\_\_\_ beta and IL-8 and at the bottom for cytokines TNF-alpha, IL-beta and IL-6 and if we pre-incubate the cells we can get a 99 percent shutdown of the chemokine and cytokine response. And I haven't shown the data for clarity but we can actually add the dendrimer glucosamine up to eight hours off the LPS and still get a substantial response. So this seemed to deal with the problem of the pro-inflammatory response.

Now the second thing that's important in this is angiogenesis and we became convinced that one of the reasons why people have failed in the past is that there is so much redundancy in these biological mechanisms that you need to think about interfering with both sides of the equation rather than just with one side of the equation. And if you think about angiogenesis and think about fibroblast growth factor 2, this is another beautiful example of polyvalency in action in biology.

So what you have is you have fibroblast growth factor which binds to cell surface heparin sulfate proteoglycans in a polyvalent manner. And only when this concentration and this complex is formed, is it able to bind to its receptor and trigger the intracellular response.

And so what we did here is that we made a sulfated dendrimer which was able to compete directly as an antagonist with this fibroblast growth factor 2 complex. And the sort of experiments that we did this in, it was a human placental angiogenesis assay, you take a placenta within six hours of birth, put it in culture for up to 30 days and you get these beautiful new blood vessels that form. If you incubated it without compound there was a reduction in new blood vessel formation of about 50 percent and we went on to show that this was due to an inhibition of fibroblast growth factor 2 mediated angiogenesis, but VEGF mediated angiogenesis was left untouched. We thought it was probably a bad idea to interfere with all of angiogenesis at a time when healing has to take place.

So we went on from there and said, "well let's actually do an experiment in an animal model where we interfere with immune modulation in a controlled manner and we interfere with angiogenesis in the controlled manner. But let's put the boulders at the top of the waterfall rather than put them at the bottom of the waterfall." And we met up with Pankow in London and looked at his model of glaucoma filtration surgery – I realize this

audience would know all this so please forgive me – but in essence what that consists of is putting a tube in creating a bleb. And the problem you run into is that the tube actually scars over and, therefore, the surgery fails. And what we've got here is a rabbit with the tube in place, and here with the bleb shown. And we know it's working because the eye is nice and moist. And what we did was a study in 30 rabbits over 30 days and in that study we were able to increase the success rate of the surgery from 30 percent at day 30 up to 80 percent at day 30.

When we looked at the histology of these samples – this is a H&E stain – you can see the conjunctiva and the cornea and you can see here the tube with really what is quite extensive scar tissue formation in the control. If you do the same stain for animals that were treated with dendrimer glucosamine with the dendrimer conjugates what you find is that there is very, very little scar tissue formation and the general morphology of the tissue is well maintained. This is a Masson's trichrome stain for collagen tissue, and again, you can see the collagen as you would expect in the conjunctiva and in the cornea and you see scar tissue here around the tube. But if you do the same experiment in the dendrimer conjugate-treated animals you've got very little scar tissue that's formed around the tube.

And then in this one here what we have is a picrosirius red stain under fluorescence microscopy. So this is the back of the eye, this is the front of the eye, and again what you can see here is you can see the collagen in the conjunctiva and in the cornea and you can see how well preserved the normal architecture is around where the tube has gone in.

So this is – we think this has a potential fall beyond the eye – but of course the model was a very nice way of looking carefully and precisely at this difficult issue of scar tissue formation. One could much more provocatively suggest that actually you could think about the back of the eye in the same simple manner in that there were diseases, this is proliferative diabetic retinopathy, where the core problems are actually inflammation and angiogenesis. And what is interesting about dendrimer based constructs is that actually dendrimer based constructs will accumulate selectively up to 50-fold in areas of inflammation compared to normal tissues. And certainly if you work with anionic dendrimers, to date we have yet to poison any animal to which we've given them with very, very large amounts of these compounds. I'd like to thank NIH, Wellcome Trust, the \_\_\_\_\_, the \_\_\_\_\_ Katz Foundation, \_\_\_\_\_ Trustees, Alcon Research Institute, \_\_\_\_\_ trustees for funding for the work that's been reported today. Thank you.

**Ritch:** Are there any questions from the audience? Professor Shaunak, I have a question. In terms of the retinal applications, have you considered trying to use this for treatment of proliferative vitreoretinopathy? I'm not sure if you've discussed this.

**A:** No, I was really just trying to raise the idea that in any situation where you think of inflammation and angiogenesis there's absolutely no reason why this kind of approach shouldn't be applicable whether it's topical or systemic.

**Q:** So if you—I don't know how you apply these substances—but if you had an air filled eye, say, and a giant tear in the retina would you be able to spray it onto the retinal surface or how would you deliver it?

**A:** There is no reason why you shouldn't be able to. I mean, in these particular studies, we applied them locally and we gave them systemically. Because one of the things that we underestimate and at least from infectious diseases perspective—which is where I come from—is how profound and important the systemic inflammatory response is even

when the point of injury is quite local. So I suspect that what one's looking at, at least in the early studies, is always to apply it locally and systemically to make sure you don't get a false negative. Once you've gone that far you can then start looking at \_\_\_\_\_ type approaches.

Yes sir.

**Q:** I had a question about the rabbit studies. How did you assess success or failure in a rabbit that has normal IOP?

**A:** We looked really for scar tissue formation in the rabbits, of every single eye section. Professor \_\_\_\_\_ is here and actually did those experiments and he might want to comment on that specifically.

Do you want to add a couple of words on the (inaudible)?

**A:** In our model obviously IOP is relatively inaccurate. And so we didn't use masked reading of the bleb appearance, whether it's raised or flat, that's the definition of failure or success. And that's ratified by a masked...

**Q:** So you have no idea what the outflow facility is?

**A:** We did not measure the outflow. We did measure IOP but we didn't do outflow.

**Q:** This is just a small point. I think what working with Sunil has shown us with these effects on inflammation as you all know, inflammation has reared its head very high in a lot of diseases and I think it's re-energized our interest in the suppression of inflammation in preventing scar tissue because really we're used to using steroids which don't really do the whole trick. But having seen the sort of effects that you can get when you properly suppress inflammation that certainly made us very interested again in that.

Dr. Sieving?

**Q:** Why is the dendrimer approach better than a blocking antibody?

**A:** I'm not sure it is necessarily better. I think the issue with monoclonal antibodies or antibody fragments is that we may be generating effects that are fundamentally different to the ones that we expect and also we tend to have affinities which are very high. So for example, if you take the experiments that have been going on in London with the anti-CD28 antibody, very unusual, very unexpected things have happened. The advantage of using dendrimers in the manner we have is that we know that the effects can be reversed within eight to 12 hours. So if you're going to manipulate, if you're going to use new immune modulators, not only can you make them selective but you know that there is an element of reversibility and you can't at the moment build into antibodies.

Dr. Shaunak, I have one last question. Oh I'm sorry, Dr. Richards.

**Q:** So to what extent do you get these taken up by cells rather than directing it things that are external to the cell.

**A:** That's a very good question. I mean there is some evidence that if you change the structure of the dendrimer you can actually get it to be taken up by a cell. Now classically you would expect the macrophage, for example, to take it up as a simple large carbohydrate structure. From all the work we've done, we can't find any evidence that that's detrimental to the macrophage per se. Take up into other cells, people have been working in oncology and looking at malignant cells, is potentially a mechanism by which you could use it to deliver an anticancer drug to a molecule. So I think you have

to choose your cell type very precisely and what you want that cell type to do. But one of the huge advantages of anionic large molecules is that they seem to be fundamentally different to cationic molecules. So for example in a previous life in the HIV and AIDS world I managed to give ten thousand times more of a linear anionic polymer to humans than have ever been given for a cationic polymer.

**Q:** Just one last question. Have you contemplated using this approach to suppress immune response?

**A:** Well one of the concerns we had—and I didn't have time to go into this--is that clearly you could make these molecules so immunosuppressive that they become dangerous. So what we built into this particular molecule was the ability to inhibit chemokines and cytokines but it didn't prevent dendritic cell maturation. So the huge advantage was that we found that by interfering with only a particular part of toll-like receptor 4 we could allow beta interferons production to precede and dendritic cell maturation to occur. So I think the feel that this offers the potential for is a selectivity in immune modulation. But you have to define what component of the immune system you want to inhibit and why you want to inhibit it, which is why defining the end game, the clinical problem, becomes so important at the start of the study.