The evolution of the danger theory
Polly Matzinger
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Author(s): Polly Matzinger [1]

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What recent research that uses your danger theory have you found particularly interesting?

Recently, I attended The RODIN conference on hemophilia in Amsterdam (18 November 2011, The Netherlands) and realized that the hemophilia community has generally accepted the danger model and is, and indeed has been for a couple of years, clinically testing it in a way I did not previously know of. In 2001, the hemophilia community seemed to catch on to the model. You see, the danger model makes the prediction that the immune system will respond to molecules that enter the body and do damage, causing the damaged tissues to release immune-stimulating alarm signals. A side consequence of this is that, if we give the body a molecule at the time that something else has caused damage, the immune system will associate that new molecule with the (unassociated) damage, and respond to it. When someone bleeds, it is a sure sign that damage has happened somewhere. So, when clotting factors, like Factor VIII are given as treatment for hemophilia at the time of bleeding, this is effectively the introduction of a foreign substance at the time alarm signals are being released; hemophiliacs are of course bleeding because they do not have clotting factor and therefore the infused clotting factor is foreign to them. It is not then surprising that the hemophiliacs start producing antibodies to the clotting factor, so-called 'inhibitors'. These eventually have to be compensated for by increasing the dose of clotting factors, which escalates to the point that the treatment becomes very expensive and then ultimately infective when the clotting factors are unable to overcome the inhibitors.

To ask if Factor VIII might itself serve as an alarm signal, Birgit Riepert's group in Vienna (Austria) tested Factor VIII's ability to turn on dendritic cells by itself, and demonstrated that this does not occur in either its native form or in the cleaved form

generated during the clotting process, thus showing that it is not itself an alarm signal. This was important to learn, as it meant that if you could give the clotting factor at a time when other alarm signals are not present, an immune response should not be raised, even though the protein is foreign.

Subsequently, Kurnik *et al.* of the Ludwig Maximilian University of Munich, München, Germany, began a study in which they took newly diagnosed hemophilia patients (who are usually boys of an average of 10 months of age admitted to the hospital) and set up a regime in which they were given the clotting factor intravenously once a week at times when they were typically not experiencing danger. For example, the physicians avoided times at which the child was bleeding or bruised, receiving a vaccine, undergoing surgery, or experiencing a cold or other illness. In this way, the investigators tried to avoid times of danger. The results of the study demonstrated that, by 2 years of age, a total of 14 out of the 30 patients in a historical control group had developed inhibitors and only one of the 26 patients in the test group had done so [1] . I first learned of this at the RODIN hemophilia conference. First, I think this is a wonderful result and am excited that a larger trial is now being planned. Second, I think that waiting until an infant is 10 months old to start the treatment is too long. However, the problem is that hemophilia, despite running in families, is usually undiagnosed until the time at which the parents admit their child to the hospital, thus starting earlier isn't always possible. However, in situations where hemophilia status is known, I would suggest starting treatment from birth. Why wait until bleeding (and the attendant alarm signals) has already happened? In addition, I think that the possibility of oral administration should be explored, as intravenous administration inherently involves a small amount of damage, and also because parent compliance with the study might remain higher if the child does not need to be hooked up and given something intravenously each week.

The community that I thought would be the first to accept the danger model was the transplant community and, on the whole, they have. However, the problem is that it is really difficult to perform an organ transplant without doing damage; in comparison, the hemophilia community can give clotting factor without doing a lot of damage. It is really just a different form of transplant, and one in which we can finally test the model in humans.

In what ways has your theory evolved since its original inception?

For a long time the model did not evolve; however, there were certain things that did not fit with the model. When things do not fit with a theory it bothers me, because either the theory is wrong or, as it turns out in this case, some of the things covered by the theory should not have been included. This was something I did not know at the time because I am not a Doctor of Medicine. In the past, people would give me a medical case or example of some kind that I could not explain within the theory. Some of those examples turned out to be wrong, but sometimes they were right and I just did not have

enough knowledge to understand why they did not fit with the theory. One example of this is graft-versus-host disease (GvHD). In experimental situations, you can take a perfectly healthy mouse that is a cross between two strains (say, A and B) and infuse into it the T cells from one of those strains (e.g., strain A). The T cells are perfectly healthy and, aside from infusion, that is all you do to them. In these cases the 'A'-type parental T cells become activated and kill the A × B mouse. The self/nonself model has no problem with this, as strain A T cells 'see' the strain B molecules in the mouse as foreign and kill it. With regard to the danger model, people asked why this was occurring. Where are the danger or alarm signals in that healthy mouse? At that point I did not have an answer but equally did not want to throw out the entire model for that reason.

One day I was teaching the danger theory to new Doctors of Medicine at the NIH. After my talk, a student who studied GvHD came up to me and said that the model helped him understand something he had never understood before. I, of course, asked him to explain it to me. It turns out that there are certain organs that are particularly susceptible to GvHD; the gut and the skin, and it is not clear why. Alongside this, it was understood that in animal colonies that have been getting cleaner and cleaner over decades, GvHD has been harder and harder to induce. With germ-free mice you almost cannot induce it at all. The skin, the gut and also the lung are the organs that are most in contact with the external world, commensals and pathogens; they are organs that are therefore always under some condition of damage and danger. In the perfectly normal healthy mouse, there are a few activated dendritic cells in the gut and skin that are responding at any particular moment to some organisms. In germ-free mice, you have a healthy mouse but with no commensals or pathogens. This means no alarm signals and therefore no immune stimulation, and thus no GvHD in these mice.

In our discussion, this student also suggested that the theory might explain why people with vitiligo experience symptoms of the disease in the locations they do. People with vitiligo do not usually have white spots on their stomachs. They have them on their face, elbows and knees, hands and feet and anywhere they get a cut or a bruise. That made me realize that there was an aspect that I hadn't considered in the model: not only do you need damage and danger to turn on a immune response, but you need damage and danger to bring the immune response to the right place! People with vitiligo have T cells that are activated against their own melanocytes. However, these only travel to places in which damage is experienced, such as the hands and feet and face, and not places like your stomach that is frequently protected from day-to-day damage.

This is an aspect of the evolution of the model that I should have realized earlier because I'd been following the work on the trafficking of immune cells that explains how they leave the blood and lymph nodes for tissue when the endothelium of the blood vessel becomes activated. In the case of vitiligo patients, the self-reactive T cells were directed only to those areas where damage was occurring, and it took a medic to

explain this evolution of the model to me!

How has your model evolved with regards to allergy?

The most important thing that the model did not originally cover was asthma and allergy. Yet it covers all kinds of other things, such as why mothers' immune systems do not kill their fetuses, why your immune system does not turn against you at puberty when you change and why newly lactating breasts do not experience severe immune reactions to the newly produced milk proteins.

Asthma and allergy bothered me because it is a whole area of immunity. It took years before I realized that the question that allergists ask about allergens is actually two separate questions. One of which is covered by the danger model and the other of which is not. The question they ask is, "what is it about some proteins in an organism like house dust mite that cause some people and not others to make IgE?" House dust mite, for example, has two main allergens, Der p 1 and Der p 2, yet has thousands of other proteins that are not allergens. So what is it about 'allergenic' proteins that cause some people and not others to make IgE? It turns out that this is actually two questions. One of these is, "what is it about those proteins, compared to the others in house dust mites, that cause people to respond?" The second question is, "why do some people make IgE?"

The first question can be answered by the danger model and the answer is simply that the proteins that are allergenic are allergenic because they are immunogenic. In terms of the danger model, this means that they are either themselves causing damage, they are packaged with something that is causing damage or they mimic endogenous alarm signals. For example, Der p 1 is a protease that attacks the surface of lung epithelium and also the surface of B cells. Bee venom is also not innocuous. Other allergens, like seed proteins, may mimic alarm signals [2] .

The second question concerns why some people make immune responses while others do not. I think this is the wrong question. I think that most people actually respond to allergens. We just do not notice it because they do not make IgE. If you were to test the nonatopic siblings of atopic children, you would probably find that they were also making immune responses to the same allergens as their allergic siblings. However, they would be producing IgG and not IgE and thus would not experience allergic reactions. Therefore the question should be, 'why do some people make IgE while others make IgG?' The answer to this question comes from a different aspect of immunity, one which the original danger model was not intended to cover. The danger model was designed to answer the first question the immune system needs to ask when faced with something. Namely, 'do I respond or not?' However, once you respond, there is a second question to ask, 'What kind of response do I make?' This second question is

the one that I think allergy falls into. Why IgE not IgG? Why $CD8^+$ T cells to some pathogens and antibodies to others? Why mixtures of cellular and humoral responses to yet others? How does the immune system determine what kind of response it is going to make? I struggled with that question for approximately 10 years and had no idea. Nobody has yet made a general model of immunity that deals with this question: the control of effector class. But it turns out that living and working with the danger model for 13 years finally gave me a clue. The danger model says that it is a tissue that controls whether you turn on an immune response, by sending alarm signals. It is also a tissue that induces tolerance by allowing its antigens to be presented without alarm signals. Perhaps, therefore, it could also be the tissue that determines the class of immunity.

Students are taught that the immune system suits the class of response to the pathogen it is fighting, For example, you produce IgE in response to worms because they are worms, you make killer T cells to a virus because it is a virus. I do not think that anymore. In fact, in a recent paper I outlined a model based on the idea that you tailor the class of response to the tissue in which it occurs, rather than the pathogen you're fighting [3] .

In living with the model for all these years, I have now realized that it is much, much bigger than it originally was and can explain not only question 1 but also question 2. So how does that apply to allergy?

People have been trying to figure out what is wrong with the immune system in people that become allergic. I think that we have been asking the wrong question. I think that rather than looking at the immune system in children who have asthma, we should be looking at their lungs. I am not the first person to say that. Others have suggested that people with asthma may have abnormal hyperactive lung responses. But the real question is why.

The danger model offers us several possibilities. One, of course, is that there is a mutation in some kind of lung function that causes the lung to malfunction and send the wrong kind of tissue signals to the immune system. By that I mean that each tissue has certain kinds of immune responses that it likes and dislikes. Tissues are delicate and have designated functions to perform; they cannot allow themselves to be overly infiltrated with immune cells that might destroy them or their function in the process of fighting infection. Each tissue has a type of immunity it likes. For the gut, it is pretty clear that this is IgA. For example, the response to rotavirus in the gut generates IgA. The gut does not like the kinds of immune response that the skin makes to tuberculosis (Th1 or DTH). It likes IgA. The lung, like any other tissue, has a way of communicating with the immune system to ensure that an immune response in the lung is the kind that can eliminate a pathogen without eliminating the lung. That communication, of course, is controlled by genes, and genes can have mutations. Therefore there may be people

with mutations in this communication process. These people could be prone to asthma. Another possibility is that there are toxins in our environment that affect the lung in such a way that it becomes unable to communicate properly with the immune system. One of those toxins that is particularly prevalent in the west is chlorine. Olympic swimmers, for example, have a high incidence of asthma.

Are you talking about the pool chlorine hypothesis?

Yes. When I was a kid in Los Angeles, USA, and my mother told me to clean my aquarium, I learned fairly quickly that you need to let the tap water stand for 3-5 days before your fish can go in it. The reason is that there is enough chlorine in the water to kill fish! Chlorine, after all, is a poison that we put in water to kill organisms. So I got together with an epidemiologist to ask whether the rise in the incidence of allergy and asthma that we have seen over the last 60 years correlates at all with the timing of the chlorination of water of various municipalities. The answer is unfortunately no, but there is beginning to be a hint that the rise in allergy might correlate with the advent of showers. If you think about it, people in the Western world used to have weekly baths. Now, we take daily showers, and breathe in hot chlorine. What impact might this have on our lungs or skin? Chlorine is not necessarily the only example. There are toxins everywhere now. In the plastic of our drinking water bottles, in the liners of aluminum cans, from pesticides in the food we eat and the air we breathe. I have no idea what the impact of that might be on the immune system. If you think that immunity is governed by a self/nonself discrimination, then toxins in the environment do not matter that much. However, if you think that the immune system is governed by damage and danger, then toxins in the environment could have a huge impact. So, I think that we really need to start thinking about damage to tissues as a potential instigator of allergy and asthma.

Another area that this idea impacts is autoimmunity; one of the questions people often ask is why women are more prone to some autoimmune diseases than men, such as systemic lupus erythematosus and multiple sclerosis.

In lupus, and this isn't a perfect answer, I think that there may be two reasons. One is that there is death that goes on in female bodies that does not occur in male bodies. Every month we pop an ovarian follicle. Every month, we kill off, clean up, and regenerate an entire uterine lining. That is a lot of death, damage and cleaning up to do. People who study the genes that govern death in *Caenorhabditis elegans* have found that 19 genes govern that process. Of those, 11 are involved in cleaning up and scavenging. For every gene found in *C. elegans* they find approximately five to ten in vertebrates. That is a lot of genes and a lot of potential mutations. Therefore, one reason women may be more prone to something like lupus could simply be that there are a lot of activated genes governing a process that men do not have. A mutation in a death gene could cause a normal death process to go wrong and allow the release of alarm signals. Since it occurs in cycles, this would lead to relapsing and recurring

autoimmunity.

Another possibility is that, with the exception of a few jobs where men are exposed to toxic substances, women tend to be exposed to more toxic substances than men, such as cleaning chemicals, hair dyes and cosmetics.

The course of lupus being relapsing & remitting would appear to fit with evidence & self-reports of flares being linked to menstrual cycles & then reducing during pregnancy. Could you comment on this?

Indeed, one of the things I have suggested to rheumatologists if they see a teenager being admitted with lupus, is that she should be put on the contraceptive pill and not allowed to cycle. They reply with either one of two things: a) that has been tried; or b) we cannot do that, as preventing menstrual cycles would not be very physiological.

I asked about examples where it had been tried and found that the girls were put on the pill to test this but were still allowed to cycle in the normal way. If the rheumatologists say that it is not physiological to prevent cycling, I disagree. If you think about it, for most of history women were pregnant most of the time. We were not designed to cycle. Until very recently in human history, women have been pregnant, lactating and then pregnant again. The cycling all the time could be considered nonphysiological.

With asthma, I imagine it could be difficult to study this, particularly as asthma is now frequently considered a syndrome of diseases. How might this impact on understanding in this case?

I heard a wonderful talk from Fernando Martinez (University of Arizona, USA) about 3-6 months ago. The most amazing thing I learned from him is that there are two very different forms of asthma; one of them has an infiltrate of neutrophils, the other has an infiltrate of eosinophils, suggesting that these are probably two very different diseases. The infiltrate gives you similar symptoms but the causes are different. If we are really going to understand asthma, it is important that we do not mix these two types of people up when we undertake clinical trials. Of course, this leads to another frequently discussed question. That of individual medicine or personalized medicine. If you run a clinical trial and find that 5% of people respond, you usually say that the results are not statistically significant and the product must therefore be a bad product. In my mind that is crazy. It is a great product for that 5% of people. For asthma, we might see that the split could be 50/50, and as long as we keep mixing these asthmatic groups up, we aren't going to understand this disease.

How has the danger theory revealed the directions that need to be taken for

future drugs & transplantation protocols?

We can see this in several ways. First, by looking at current drugs. For example cyclosporin A (CsA) is an immunosuppressant that has kept many transplant patients alive. However, it has kept these people alive at great expense and at great risk of infection, and at the risk of preventing tolerance, because it blocks the wrong signals. I'm not the first person to say this. Sir Roy Caln was a liver transplanter in Cambridge, UK, back when CsA was first discovered. He showed, in a rat model of liver transplantation, that transplanted livers could induce tolerance even without immunosuppressants, and that this tolerance would allow a liver-transplanted rat to accept a skin graft from the liver donor. However, if Caln gave CsA at the same time as the liver transplant, he prevented the induction of tolerance. This was done in the mid 1970s and should have woken people up to the dangers of CsA. Alan Kirk later published a study describing kidney transplants in six monkeys that were given anti-CD40L to block signal 2 [4] . He gave the blocking antibodies for 5 months then stopped. None of the monkeys rejected, and by the time he published some of the monkeys had carried their grafts for 6 years and still had not rejected. This shows that the details matter. We should be mimicking the body's own ways of inducing tolerance, with signal 2 blockers that can be given for a short time, rather than signal 1 blockers that must be given for the life of the patient.

Second, we should look at limiting the damage done during transplant procedures. One day I was in Holland talking about danger, and Jan van Rood - who founded Eurotransplant - jumped up in the back having realized why completely unmatched living donor kidneys do better post-transplant than matched cadaver kidneys. When you use living donors for transplantation there are a number of benefits with regards to damage. First, you do not have to perfuse the kidney and therefore you do not get reperfusion injury. Second, you do not have the time for injury and damage to occur while it is being transported around the country between hospitals. In living donor transplant, the donor and recipient are normally in the same operating theater and the kidney is taken from the donor and transferred directly to the recipient. This results in far less damage, far fewer alarm signals and a much greater tolerizing capacity. So, creating minimal damage and blocking signal 2 would be the things to do to get better transplant results.

Third, we could try blocking the alarm signals. Walter Land, ex-head of experimental surgery at the Medical School, Munich, Germany, was one of the first surgeons to understand the danger model. In a way, he discovered it before I published it. He had run a clinical trial in kidney transplant patients to try to enhance immediate kidney function. Sometimes, for unknown reasons, a transplanted kidney never starts functioning. Land hypothesized that maybe reperfusion injury and the attendant oxygen radicals damaged the kidney so much that it could not function. Therefore, he decided to give a single shot of the oxygen free radical scavenger, superoxide dismutase (SOD)

at the time of transplant. Forty-four patients got the SOD and 44 did not, but otherwise they were treated completely the same. It turned out that the SOD made a slight difference in immediate function but not a great one. They followed the patients anyway and, a few years later, Land was approached by his computer analyst who had found the amazing result that the 44 patients that got the shot had experienced almost no acute rejection episodes, compared to the patients that did not get the SOD. Land and his institute carried out more studies in rats looking at the effect of SOD and found that it was highly effective, and started telling the world that immunity was greatly influenced by tissue damage.

Unfortunately, because a single shot of SOD is an inexpensive treatment, the clinical trial that would be needed to take this treatment to the clinic would be so much more expensive than any potential profit, no pharmaceutical company is likely to pursue it. There are many such treatments discovered by researchers. These are the kinds of treatments for which the clinical trials ought to be funded by a public health system, such as the NHS in the UK, or indeed insurance companies in countries like the USA. It would ultimately save these groups tons of money in long-term follow-up of these patients, and save the patients a lot of pain; but it is unlikely to happen.

Because of the success with SOD, and with living kidney donors, I think that we should focus on minimizing damage and blocking signal 2. Blocking the alarm signals would be a difficult proposition, as there are too many of them. Anything that is normally found inside a cell, and not outside, can be an alarm signal and many have already been found, such as DNA, RNA, ATP, uric acid crystals, hyaluron break-down products, heat shock proteins, and (my favorite) mitochondria. Right now, I'm not sure what kinds of drugs we would try to use to block all these.

Following on from allergy & asthma, how do you think the hygiene hypothesis fits with your theory?

There are two aspects to this. One is the one that everyone talks about, which is that it is good to grow up dirty. There is a whole conversation that could be had about this. Such as, what about being dirty is good? Is it that being dirty is good or being too clean is bad? Is having infections good or is it that not having them is bad? This need not be the same thing! Is it because when you are dirty you get commensals at an early age that affects your gut or skin in such a way that it sets the immune system in such as way that you do not get allergies. Or is it that the commensals prevent pathogens from coming in later? Another aspect that is less commonly discussed comes up from evidence that farm children get fewer allergies than city kids. I have a farm, train sheepdogs and spend a lot of time with farmers, and I have noticed that they prefer to use well water where possible. They drink and shower with this water and as a result do not breath hot chlorine every day. They're not exposed to some of the toxins that city people use to keep themselves clean! That is an aspect of farm life that I think has not

been considered but should be.

Of course many of them spray pesticides on their fields. It would be interesting to divide farmers into organic and nonorganic farmers and look at the rates of allergy and asthma there. The organic people are probably not only growing their crops organically but living their lives a little more organically and are probably less likely to be in contact with as many toxins.

Your recent publication "Tissue-based class control: the other side of tolerance" [3] concerned the control of effector T cells. What impact do you imagine such theories might have on patient care in the clinic?

I do not think theory has much of an impact on patient care. I do not blame physicians. They do not have a lot of time, work pretty hard and usually see far too many patients each day. Even if they knew the theories that were coming around (and most of them do not), I do not think that they would put anything into effect unless something in their practice had already impinged on them to make them open to the theory. Theory can impact basic research, which itself impacts clinical research very slowly, and thus may, or may not, eventually have an impact on clinical practice.

If the danger model has made a difference in clinical practice anywhere, it has made a difference in bone marrow transplants. A GvHD expert named Riener Storb, from Seattle (WA, USA) was at the forefront of the move to make preparation of the recipient for bone marrow transplant as nondamaging as possible, for example avoiding massive radiation. Storb understood that a person who is prepared for his bone marrow transplant in a less aggressive way has a lower risk of GvHD. I do not know whether that practice was sparked by the danger model or sparked by something else and only supported by the danger model, but it is a place in clinical practice where the model has had an affect. I do not yet think it has had much of an effect on clinical transplant practice, though it has certainly had an affect on experimental transplant practice. Starting with Alan Kirk, many researchers are now working on blocking signal 2 and on doing less damage. I think that the model has the most potential impact in therapy for hemophilia, where danger-based clinical trials are beginning to occur, and for tumor therapy. However, the tumor experts do not seem to be listening. Approximately 10 years ago, I suggested that we have the capacity to clear 80% of tumors with immunotherapy, yet we are not doing it. The reason is that immunotherapy is not being properly used. What you think influences what you do. If you think about the immune system in one way you will do something different to someone who thinks about it in another way.

How do you think that tumor vaccines should be used differently?

Two of three things could change. First, most tumor vaccines are used in the same way that most antiviral vaccines are used. People get a priming shot and then a boost, and that is it. This works for vaccines against viruses but it would not work for tumor vaccines. When you vaccinate against a virus, you prime to activate the T cells specific for the virus, and boost to expand the number, after which they expand, contract a little, then go on to a long-lasting resting memory state. If the virus arrives, it does the damage that wakes up APC that in turn reactivate those vaccinated resting memory T cells. Thus, for a viral vaccine, producing a large population of virus-specific resting memory cells is great because the virally-induced damage is immunostimulatory.

However, vaccinating against a tumor is a different story. First, this needs to be a tumor against which the person still has a few specific T cells, because an early growing tumor is a healthy tissue not sending alarm signals, and therefore is constantly inducing tolerance to itself. Hoping that the tolerance is not complete, you vaccinate to increase the number of the few remaining tumor-specific T cells. Then you boost to expand this population further. Yet, even though studies using tetramers can show an increased frequency of the tumor-specific T cells in blood, the tumor is not cleared. I think there are three reasons for this.

First, after the boost, the cells do what they are programed to do, which is kill one round of tumor cells then go into a resting memory state. The killing done by a cytotoxic T cell is apoptotic; it does not cause the release of alarm signals so it does not boost the response, which consequently displays typical immune response kinetics and wanes after about 2 weeks. All those killer cells that were activated by the vaccination go back into a resting memory state. This is fine if you have killed the last tumor cell but, if not, the tumor will continue to grow. So you need to boost, and boost and keep boosting.

There was a clinical trial, which did not get the recognition it should have, showing that this works. Maurizio Bendandi (Pamplona, Spain) used a vaccine for people who have B cell lymphoma. This is an individualized tumor-specific vaccine made by taking the antibodies produced by the patient's lymphoma cells and coupling them to keyhole limpet hemocyanin. This produces the vaccine, which is specific to the lymphoma and individual to the patient. The original protocol, which was invented by Ron Levy at Stanford, is better than most, as patients get five injections, rather than the standard two. Although this works on some patients, many relapse. Bendandi managed, after 3 years, to get board approval to keep boosting with the vaccine. Each patient was their own control. Each had relapsed previously and was treated again with chemotherapy. Vaccination began after 3 months when their immune systems had recovered. Bendandi kept vaccinating them month after month, and got an amazing result! Eighteen of the twenty patients had not relapsed by twice the amount of time their first relapse took place [5] . Unfortunately, one subject died and after writing to Bendandi, I learned that they had run out of his vaccine at 2 years, and the patient died at 2.5 years. This approach certainly looked like it worked but for some reason it has not gotten much

attention.

The danger model supports this approach, as it says you have to keep boosting. The second thing you have to do is bear in mind, as with vitiligo, that even if you have an activated immune system it will only locate to certain places. So you need to do damage or something to the tumor to direct the activated cells there. Otherwise you can boost and activate all the tumor-specific cells but if the endothelial cells in the blood vessels are not activated the lymphocytes are not going to extravasate and reach the tumor. The third thing, and this part is difficult, involves the new part of the danger model. The tumor is a tissue and tissues have ways of communicating with the immune system so that a local immune response clears a pathogen without destroying the tissue itself. Thus a tumor will also have mechanisms to prevent immune-mediated destruction. When people tell me that their tumor is very immunosuppressive because it makes TGF-[beta], I bet them a bottle of champagne that the tumor is either a gut or bladder tumor. It is not making TGF-[beta] because it is a tumor, but because TGF-[beta] instructs B cells to make IgA, and this is exactly the kind of immunity that is appropriate in the gut and the bladder. What we need to do now with tumor vaccines is find a way to overcome the normal tissue signals that instruct the immune system to produce a nondestructive response. In the vaccine, you need to put in substances that are going to give a strong Th1 or delayed-type hypersensitivity killer response to kill the tumor. You need to do it in such a way that those cells ignore those tissue educating signals. We know how to make vaccines, how to boost, how to do damage, but we do not yet know much about the signals that the tissues use to educate the immune system, so we do not yet know how to overcome them.

Perhaps some sort of adjuvant could be used?

People are using adjuvants. But none of those adjuvants are designed to deal with tissue-education signals. I'm not sure what sorts of adjuvants we would want to use there. We will first need to do some basic research to get a handle on the signals that tissues use. Once we have figured those out, we will be in a position to find agents that modify them, so that we can control the response to a vaccine. In the meantime, people are trying to find adjuvants that do not do damage. I say good luck to them. An adjuvant that does not do damage is unlikely to give an immune response. Unless we start using the body's own adjuvants - meaning the alarm signals that are the result of damage. Once we get a catalog of what the alarm signals are, we should be able to start using them as nondamaging adjuvants.

What do you have coming up in the next year?

We are going in several directions. Each person in my laboratory does something different, something that they own themselves. I have found that when people own

something they do a better job. One of our recently published projects showed that the cells of the gut, in an animal missing B cells, take on their own defense [6] . They upregulate immune genes and, at the same time, downregulate their metabolic genes and no longer carry out metabolic functions. In animals that are germ free this does not happen. There appears to be a three-way conversation between commensals in the gut, B cells in the immune system and epithelial cells that line the villi. We are now trying to determine what that conversation is.

We are also working in a parasite model with *Leishmania* , to see if the location of a parasite can influence the type of immunity that it elicits. Some forms of immunity will be due to the influence of the tissues in that location, and some of them will be due to an influence of the parasite itself. This is yet another aspect of immunity that I think we have not paid enough attention to, which is that not only do tissues control the immune system but that commensals also control it. They have been with us through evolution and know how to influence our responses in their favor. My laboratory is thus beginning to move in two directions: looking at how tissues and commensals influence immunity. We are studying this in the gut and the mouth (as the mouth is easier to sample than the gut) and trying to decipher the conversations going on there.

We also have a project in which we look at the dendritic cells from various tissues to see how they differ. If we analyze dendritic cells from the gut, ear, lung and brain, we can see how they differ in patterns of gene expression and what this tells us. I want to work my way up, finding out what controls those genes before determining what the signals are that they are responding to. This won't be easy, especially because it is not something you can do in a Petri dish. We are moving to systems biology because I think that the only way we can figure this out is to ask what a cell was doing at the time that we took it out. We cannot take cells apart, put them in plastic and still get them to behave the way they would when in their original intact environment, replete with all the connections they have to other cells. We learned a lot about the interactions that control immunity by looking at single populations of cells in petri dishes, but we also missed a lot, and in order to truly understand immunity we need to study the immune system in its native habitat. The immune system is really one of those biological systems that completely represent the uncertainty principal, in that it is hard to study the immune system without changing it. The best thing we can do with the technology we have today, is it to take a cell out and as quickly as possible ask what it and its neighbors were doing at the time we removed it. By doing that perhaps we can answer these remaining questions.

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Author Affiliation(s):

[1] Ghost Laboratory, Laboratory of Cellular & Molecular Immunology, National Institute of Allergy & Infectious Diseases/NIH, Building 4 Room 211, Bethesda, MD 20894, USA. pcm@helix.nih.gov

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Polly Matzinger