

Immune-Allergic Sensitization to a Xenobiotic

Jack Uetrecht, M.D., Ph.D.

Canada Research Chair in Adverse Drug Reactions

University of Toronto

jack.uetrecht@utoronto.ca

DR. SELIGMAN: let me introduce our first speaker this afternoon, Dr. Jack Uetrecht from the University of Toronto.

DR.. UETRECHT: Thank you very much, and thank you, John, for inviting me. Okay. So I'm not going to answer the question. I'm going to leave that to Leonard. I'm going to try to convince you that we really don't know very much about these things, and we need to know a lot more about mechanisms before we can begin to answer the question.

Definition of “Idiosyncratic” Drug Reactions

- Definition depends on who is doing the defining.
- “peculiar to an individual”, i.e. does not occur in most patients.
- Does not involve the known pharmacological properties of the drug, i.e. would not include cardiovascular events associated with Vioxx.
- Several other terms - hypersensitivity, allergic, type B, etc.
- May not have a simple dose response relationship in a population but they are dose dependent!! Total daily dose may be one of the best predictors of IDR risk.

First I have to start off with a definition. So the definition of an idiosyncratic drug reaction really depends on who's doing the defining. Part of the definition is that it's peculiar to an individual but if you push the dose of the nevirapine in patients, which was done in early clinical trials, almost everybody shows it is clearly immune mediated. But if the incidence creeps above 50 percent, is it no longer idiosyncratic even if it's immune mediated? What's the most important criterion?

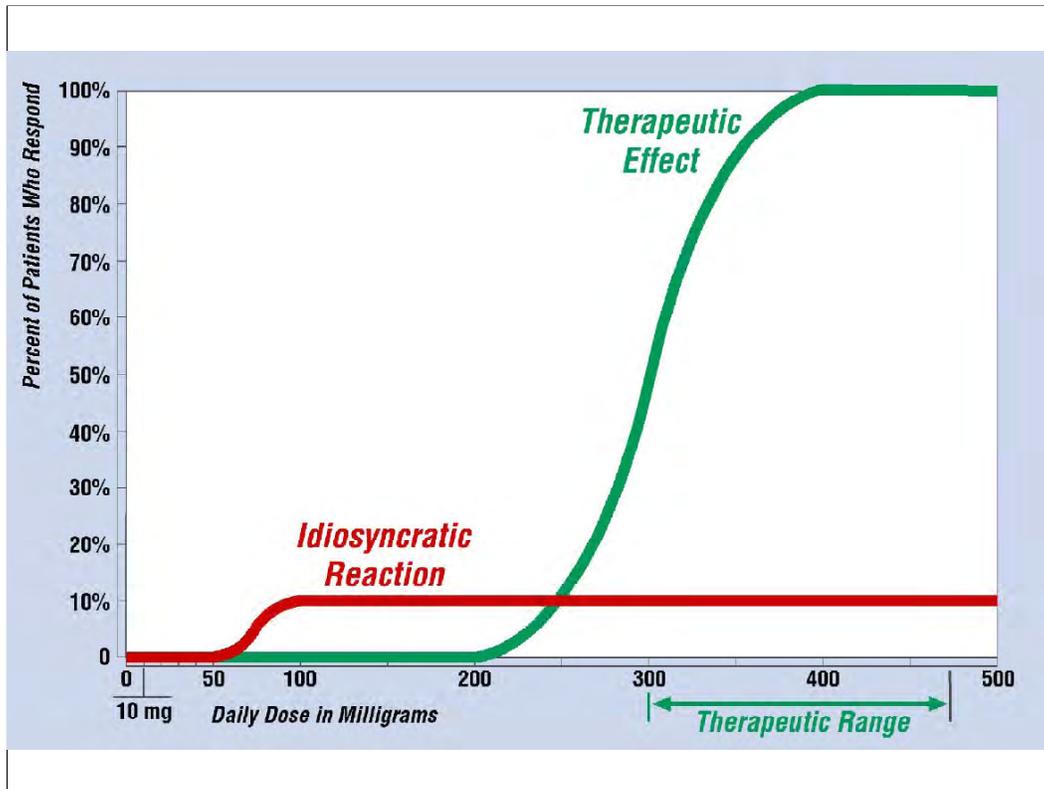
I'd like to say I'm not interested in things where the reaction involves the pharmacologic properties of the drug but certainly reactions to Vioxx or something could easily be considered idiosyncratic. Lots of other terms are used. There may not be a simple dose-response relationship but everything is dose dependent and, in fact, the total daily dose may be one of the best predictors of risk.

**IDRs may be hard to define but I
know one when I see it.**

“Hard-core pornography
is hard to define but I
know it when I see it.”
Potter Stewart, justice of
the Supreme Court



Now I was amused by something that John Senior wrote recently, something about what idiosyncratic drug toxicity is and what it isn't, and I was reminded of the words of the late Potter Stewart, Justice of the Supreme Court, "Hard-core pornography is hard to define but I know it when I see it." Likewise, an idiosyncratic drug reaction may be hard to find but I think I know it when I see it. (laughter).



Again, to emphasize and maybe I'm beating a dead horse, but even my own students read the literature and they say these are dose-independent reactions, and I want to scream. Nothing is dose-independent. They're finding nanogram per liter concentrations of drug in the drinking water because people flush it down the toilet or it just comes out in the urine, and I would say that that is totally innocuous. Nanograms per liter. But that's still 10 to the 12th molecules, 10 to the 12th molecules, Avagadro's number. If you remember what that is, it's very big and yet it's a millionfold less than the therapeutic dose, and so I don't think it has any toxicological relevance. There are plenty of other things to worry about rather than that.

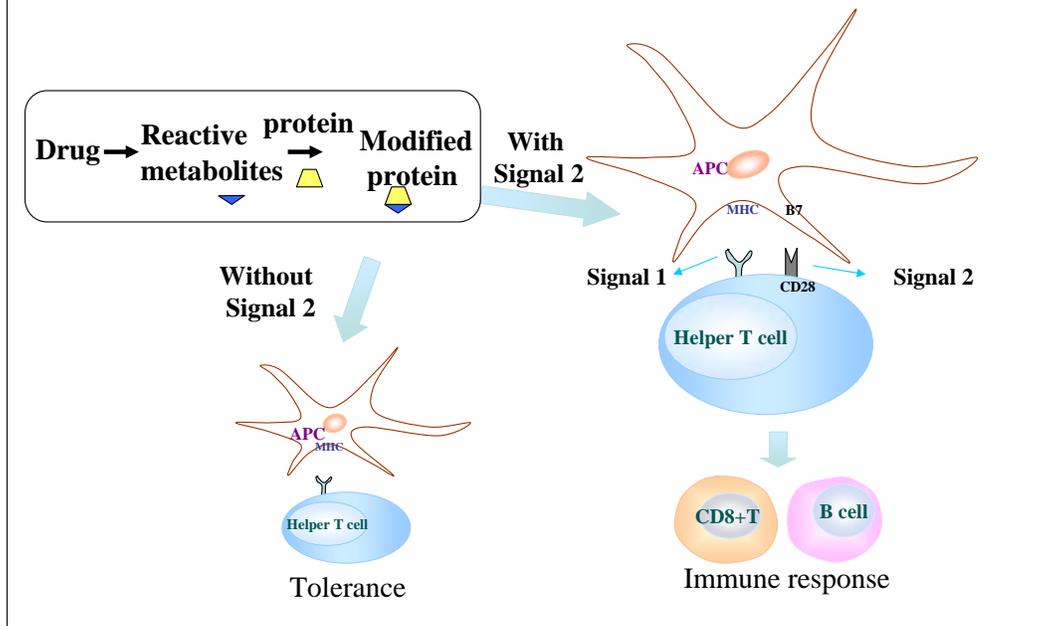
What is true is that because the mechanism for idiosyncratic reaction is different from the mechanism for the therapeutic effect, there's no reason why the dose-response curve should have its S shape in the same region and once you get to a therapeutic dose, the risk may not change. That is true, but it's not always true. In some cases, there happens to be a coincidence between the dose-response curve for the idiosyncratic reaction and the dose-response curve for the pharmacological effect.

Hy Zimmerman Categorization of Idiosyncratic Liver Toxicity

- Immune Idiosyncrasy
 - Fever, rash, and eosinophilia
 - Anti-drug antibodies
 - Rapid onset on rechallenge
- Metabolic Idiosyncrasy
 - Lack of fever, rash, eosinophilia, and antibodies
 - Often late onset
 - Lack of immune “memory”

Now Hy categorized idiosyncratic liver toxicity as an immune-idiosyncrasy and a metabolic-idiosyncrasy. Immune-idiosyncrasy was if the reaction was associated with fever, rash, eosinophilia. You often find anti-drug antibodies and there's a rapid onset on rechallenge. And so metabolic idiosyncrasy is anything else. Lack of fever, rash and negative sensibility, but a lot of immune-mediated reactions are not associated with fever and rash. So I don't like that as criteria. The onset of reactions to troglitazone and isoniazid, pyrazinamide often occur quite late and I could guess as to why that might be, but I'm not sure why it would be a characteristic of metabolic idiosyncrasy. Most important is there's often a lack of memory, and one considers immune memory as a hallmark of an immune-mediated reaction. There are memory T cells that allow for a rapid response on rechallenge.

Hapten and Danger Hypotheses



So the standard, at least for me, the mechanistic hypothesis for how a drug causes an immune-mediated reaction is: the drug or more likely a metabolites, binds to the protein, the modified protein is taken up by antigen-presenting cells, the modified peptide is presented in the context of MHC. That's signal 1. Without signal 2, you just get immune intolerance. Signal 2 is some sort of inflammatory environment that upregulates costimulatory factors and leads to active immune response.

However, we have tried to, in animals, increase both signal 1 and signal 2 and it doesn't lead to an idiosyncratic reaction. You might say, well, it just requires a specific MHC but remember, we can all respond to viruses and other things. So if there're enough different modified peptides, you'd think that that might not be a factor.

There's certainly a very important balance in the immune system. The immune system is usually very good at preventing an overreaction that is damaging, and I think that is a major factor, but how to tip that balance, I'm not sure.

Examples That Probably Represent Immune Idiosyncrasy

- β -Lactam antibiotics
- Halothane
- Tienilic acid
- Minocycline
- Phenytoin

We don't even know whether it's antibodies or T cells that mediate these reactions. So we often see anti-drug antibodies in cases of drug-induced liver injury. I suspect that those antibodies are not pathogenic, that it's T cell-mediated but nobody has ever demonstrated that. The best I've ever seen is a positive lymphocyte transformation test which means you've got some lymphocytes that respond to drug but it doesn't say that they're CD8 cells that are causing the damage to the liver. So even for the basics of what we think are immune-mediated reactions, we don't have a very good handle on.

Examples of drugs that cause reactions that are probably immune idiosyncrasy are, of course, beta lactams, Halothane, tienilic acid. Minocycline causes more of an autoimmune or classic autoimmune hepatitis. So with these reactions, rechallenge usually causes a very rapid onset which is again typical of an immune-mediated reaction.

Rechallenge in Immune-Mediated Hepatic Injury

- Rechallenge of patients to drugs that cause the classic pattern of immune-mediated damage usually leads to a rapid onset of liver injury.
- Although it is often possible to induce tolerance with an immune-mediated reaction, attempts to induce tolerance in this context are probably dangerous.

On the other hand, we have often induced immune tolerance and we certainly can do that to penicillin in terms of anaphylactic reactions.

Examples That Have Characteristics of Metabolic Idiosyncrasy

In some cases, especially with isoniazid, patients can be desensitized.

- Isoniazid
- Pyrazinamide
- Troglitazone
- Ketoconazole
- Ximelagatran??

Here are some examples of drugs with characteristics of metabolic idiosyncrasy: isoniazid, pyrazinamide, troglitazone, ketoconazole and originally ximelagatran would have been put in that category, but now there is evidence that it's immune-mediated. Certainly with isoniazid, usually you can desensitize these patients. And so rechallenge in those patients appears to be relatively safe. The few cases of pyrazinamide-induced toxicity that I know of were nasty, nasty reactions. So I would be very frightened of rechallenging those patients and I don't know if we have much data on troglitazone and ketoconazole.

So again, although desensitization may be possible, everything is risk versus benefit. Unless it was an awfully big benefit to the patient, I would be reluctant to do it.

But what metabolic pathway is idiosyncratic?

- Isoniazid hepatotoxicity is slightly more common in patients who are slow acetylators and have high Cyp 2E1 levels but the effect is much too small to be responsible for the idiosyncratic nature of isoniazid hepatotoxicity.
- Valproic acid hepatotoxicity has different characteristics and may involve mitochondrial metabolic idiosyncrasy but the mechanism is far from clear.
- Thiopurine methyltransferase is polymorphic and a deficiency in this enzyme is a significant risk factor for mercaptopurine-induced neutropenia but probably not hepatotoxicity.
- Reactions involving the innate immune system may have similar characteristics, especially lack of “memory”. It is very likely that the innate immune system is involved in most immune responses, but I have not yet seen an IDR for which there is evidence that it is mediated solely by the innate immune system.

So if we're talking about metabolic idiosyncrasy, it would be nice to know what is the metabolic pathway that's responsible for this idiosyncratic reaction? With isoniazid, it's true that it's slightly more common in patients who are slow acetylators and have high CYP 2E1 levels but the relevant risk is like 1.2. I mean it's far too small to be responsible for the idiosyncratic nature of the toxicity.

Some immune-mediated reactions have characteristics of metabolic idiosyncrasy

- Heparin-induced thrombocytopenia is clearly immune-mediated yet if patients are rechallenged after 100 days usually nothing happens.
 - Penicillamine-induced autoimmunity
 - Ximelagatran-induced hepatotoxicity; often does not recur on rechallenge but it is associated with a specific HLA genotype.
- Do some examples of “metabolic idiosyncrasy” represent autoimmune reactions?

With valproic acid, you have a very different clinical picture. There's evidence that it causes mitochondrial damage. I think that has to play some role in the toxicity and patients that have mitochondrial abnormalities to begin with, are at increased risk but it still doesn't totally explain the reaction.

Correct me if I'm wrong on this. Thiopurine methyltransferase certainly is polymorphic and a deficiency is a significant risk factor for mercaptopurine-induced neutropenia but probably not for hepatotoxicity. That's what I was able to find in the literature and if I'm wrong about that, let me know but there seems to be a disconnect between the neutropenia and the liver injury.

It turns out that some clearly immune-mediated reactions have characteristics of metabolic idiosyncrasy. A very good example is heparin-induced thrombocytopenia, clearly immune mediated, if it's mediated by antibodies against platelet factor 4, heparin complex, and yet if you wait until the antibodies disappear and you rechallenge these patients, usually nothing happens; and if it does happen, it's on the same time course as on an initial exposure.

Immune Memory in Drug-Induced Autoimmunity

- In an autoimmune reaction, by definition the antigen is still present when the drug is stopped.
- Therefore, the autoimmune reaction should continue after the offending drug is stopped.
- However, in most cases it does not.
- The auto-reactive T cells must be either deleted or anergized.
- This would eliminate immune memory.

Certainly a lack of memory could be explained by the innate immune system which doesn't have memory. I once proposed that that might be responsible for some of these types of reactions that don't have memory, but the more I think I know, I've not seen any evidence. I have the feeling that the innate immune system and the adaptive immune system are so intertwined that they never respond independently of one another. If you have one arm of the immune system responding, the other arm is involved in some way but again, we don't have any good data. So prove me wrong.

Penicillamine-induced autoimmunity, and I'll talk more about that in just a minute. And Mary Anne or Jerry will have to correct this, it looks like it wasn't really rechallenged but some patients had an increase in ALT and when given the drug again nothing happened. That could be more like isoniazid but yet, as was presented here last year, it looks like this is an immune-mediated reaction.

And the question is why is there no immune memory in a clearly immune-mediated reaction? And, is it because some of these things represent autoimmune reactions? And, why would that be?

D-penicillamine Induced Autoimmunity in BN Rats



20 mg/day
→
~3 weeks

~50-80% incidence

- Anti-nuclear antibodies
- Skin rash
- Immune complexes in the kidney
- Swollen, red arthritic limbs
- Weight loss
- **Hepatic Necrosis**
- **No decrease in delay on rechallenge**

Well, if you have a drug-induced autoimmune reaction, when you stop the drug, by definition, the antigen is still present because it's an autoimmune reaction, and yet usually when we stop the drug like procainamide or hydralazine, (I'm showing my age because these are drugs that aren't used very much anymore) usually the autoimmunity clears up fairly rapidly. Sometimes it doesn't but usually it does. So you must have gotten rid of the memory T cells. And, of course, this would eliminate the immune memory. Let me digress for a moment. I will get back to people in a minute. It does have some relevance. There's a nice animal model in which if you give penicillamine to Brown Norway rats, they develop autoimmunity, and it includes hepatic necrosis and if you allow the animals to recover, stop the drug, allow the animals to recover and rechallenge them, they again develop autoimmunity but it takes just as long as it did the first time. The incidence is only 50 to 80 percent. It's not 100 percent.

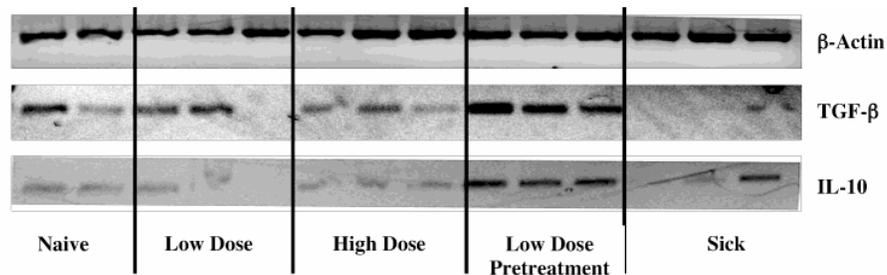
Dose Dependency of Penicillamine-Induced Autoimmunity

- Despite what is often said, all effects, including idiosyncratic drug reactions, are dose dependent.
- The dose required to induce the syndrome is 20 mg/day (incidence 50-80%).
- A dose of 50 mg/day does not increase the incidence.
- A dose of 10 mg/day for 2 weeks induces immune tolerance to the 20 mg/day dose, which can be transferred to a naïve animal with spleen cells.

Now again we get back to dose dependency. This has a very interesting dose dependency. It takes 20 milligrams a day to induce this reaction but not all the animals respond, and this is sort of strange because it's a highly inbred strain of animal. So they're virtually genetically identical, and they all have the same environment. They're all in cages in the same animal facility and yet only 50 to 80 percent of the animals respond. If you increase the dose, you don't increase the incidence. So there's no dose dependency.

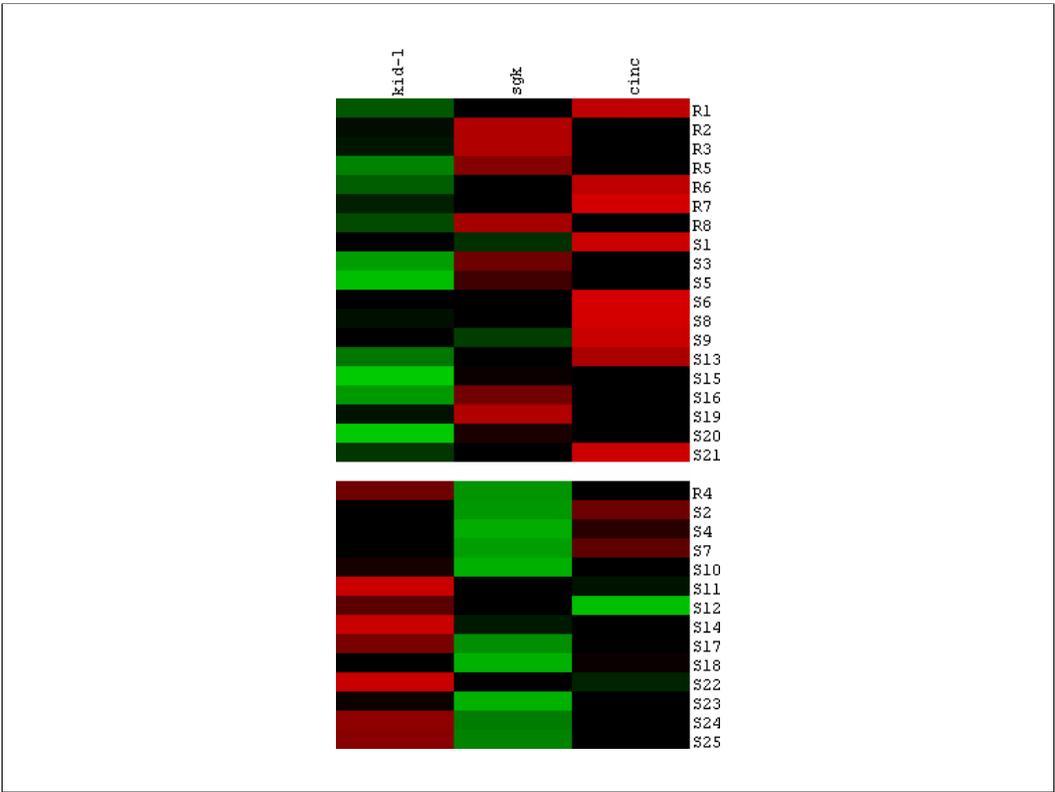
Well, that's not true either because if you go down to 10 milligrams a day, you induce tolerance and that tolerance is immune tolerance. It can be transferred from tolerant animals to naïve animals with spleen cells. Furthermore, if you rechallenge these tolerized animals, you get an increase in TGF-beta and IL-10. So apparently T-regs produce cytokines and decrease the immune response.

Cytokine Production by Tolerized CD4⁺ T cells



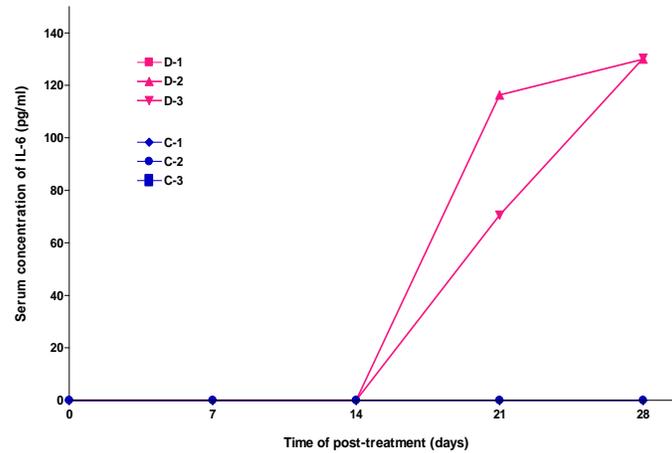
So it appears as if the decision as to whether to mount an autoimmune response occurs very early. If we give one dose of misoprostol, which has a half life of about one hour, at the very beginning of treatment, it prevents the reaction, even though it takes three weeks for them to develop autoimmune symptoms.

So we did a microarray study and the animals separated into two different populations. We were trying to determine what gene changes were associated with whether they responded with autoimmunity or not, and a weird thing happened. So again, there are these two different patterns that are distinct. All the animals in this group but one developed autoimmunity. Only one of them didn't. However, in the other group, about half of them did and half of them didn't.



So there's something going on here that didn't lead either to a clear separation nor a clear mechanistic understanding of what was going on. We did this a couple of years ago, and we sort of dropped it. We didn't know quite what to do with it.

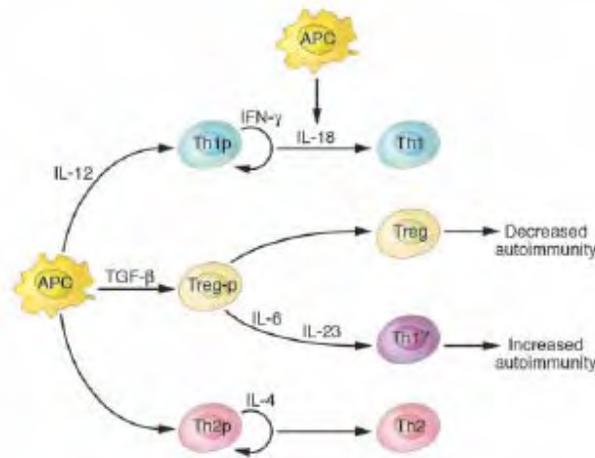
Serum IL-6 in Penicillamine-Treated Animals



Very recently, we looked at IL-6 levels, and we had three treated and three controls. Two of the three animals developed autoimmunity and in both cases their IL-6 went up and then the animal that didn't develop autoimmunity, their IL-6 didn't go up, and we've done a couple of studies since then, and this seems to be consistent.

There is a “New” Kind of T Helper Cell - Th17

I. Gutcher and B. Becher, J. Clin. Invest. **117** (5), 1119-1127 (2007).

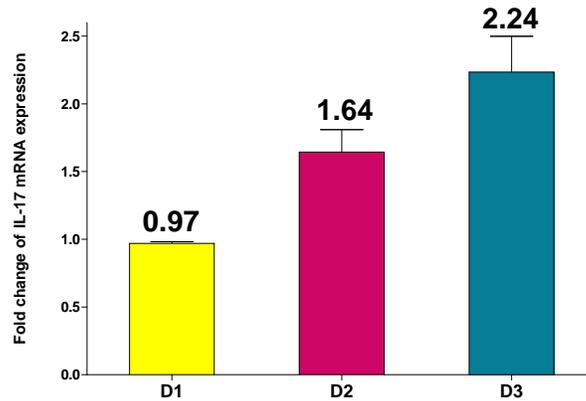


Now it turned out that there's a new kind of helper T cell. The standard paradigm is a couple of decades old, and I hate the word paradigm. It's a good word that's been misused. People say I don't believe in homeopathy because I work in a different paradigm. No, it's because homeopathy is wrong, not the paradigm. (laughter). So the standard helper T cells are Th1 that help cell-mediated responses and Th2 that help antibody-mediated responses and this is associated with the classic, gamma interferon, and this is associated with IL-4. Those are the classic cytokines associated with Th1, Th2 cells.

Now this paradigm clearly is not complete because, for example, diabetes – the NOD mouse is a cell-mediated response but if you give gamma interferon it protects. So there are all sorts of holes in this Th1, Th2 paradigm.

A new helper T cell, they skipped Th3 to Th16 to Th17. It's called Th17 because it produces IL-17, and it turns out that if these precursors to helper cells are simulated by TGF-beta, they can produce T regs which have been very popular for the last several years because they've down regulated immune responses. They keep them from getting out of control, but if there's a lot of IL-6 around and TGF-beta, you get these Th17 cells and these cells are associated with autoimmunity, things like Crohn's disease, multiple sclerosis, et cetera. And the first paper I can find on Th17 cells is 2006. So this is pretty new stuff. It's believed at this point in time that only Th17 cells produce IL-17 but again it's early days. So I never trust what they tell me.

mRNA expression of IL-17 in splenic CD4+ T cells in penicillamine-treated animals



So in these animals, there's no ELISA kit yet for rat IL-17. So we had to go to quantitative PCR. This is the animal that didn't develop autoimmunity, and there's no different from control, but the two animals that got sick had an increase in Th17 or I'm sorry, IL-17 mRNA.

So the question is does this have implications for human liver disease. Are some of these idiosyncratic hepatitis? Are they autoimmune in nature? Now I'm not talking about the classic idiopathic autoimmune disease where you have specific antibodies that are characteristic, just autoimmune in a more general way, meaning that is against self, would that explain why there's no memory, why it often occurs late because again from experience, with drugs that cause a Lupus-like syndrome, like procainamide, hydralazine, it often waits a year or more before the onset of these autoimmune reactions.

Likewise, with some of the most severe reactions, the patients get worse after you stop the drug. Why does it continue like this? Well, if it has autoimmune characteristics, that might be the explanation.

Summary

- There are examples of idiosyncratic drug-induced liver injury that have clear characteristics of being immune-mediated.
- The evidence for metabolic idiosyncrasy is weak.
- Some cases of liver injury that have been classed as metabolic idiosyncrasy may also be immune-mediated.
- The characteristics of some of these reactions may be related to a type of autoimmunity (not necessarily classic autoimmune hepatitis).
- Such liver injury may be mediated by Th17 cells.
- This hypothesis should be tested in humans.
- If Th17 cells are involved it might provide biomarkers to predict toxicity or diagnostic tests.

So in summary, there are examples of idiosyncratic drug-induced liver injury that have clear characteristics of being immune mediated although there's still people that disagree that they're immune mediated, and we don't have any absolute proof that they're immune mediated but I think the characteristics strongly suggest that they are.

The evidence from metabolic idiosyncrasy is weak. I'm not saying for a minute that it doesn't exist but I'd sure as heck like to have better evidence that it exists. And again it's a matter of how often.

Some of the cases of liver injury that have been classed as metabolic idiosyncrasy may also be immune mediated, and the characteristics of these reactions may be related to some type of autoimmunity, not necessarily classic idiopathic autoimmune hepatitis, but it's still autoimmune in nature. And such liver injury may be mediated by Th17 cells. And we really need to test this in humans, and so I'm appealing to anyone who might have samples that we can test this hypothesis. I talked to the hepatologists in Toronto, and they said, well, there isn't one person that's sees these sort of patients. It may be years before you get a good case. So I would really like to have samples, but well frozen serum should do, just to look at Th17.



IL-6, as I'm sure you're all aware, is a common cytokine that associated with the acute phase reaction and that would not be diagnostic, but I think IL-17 would be diagnostic and it would also be interesting to look at IL-23 and some other cytokines associated with these cells.

If this hypothesis tends to be correct, and I'm not saying for a moment that all drug-induced liver injury is mediated by these cells, it could provide some biomarkers to predict toxicity or some diagnostic tests, and I thank you for your attention and this is just my group.