Health Effects of PCB's: The Immune System

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Thank you very much, Dr. Nohara. I would like to thank you and the organizers very much for inviting me to this very interesting place and this symposium. I have been asked to talk about effects in of wildlife populations and humans of TCDD, PCB.

There is a whole range of chemicals that affect the immune system, such as environmental chemicals, drugs or natural toxins. Some of these chemicals have in common that they act through the thymus; thymus involution is the morphological hallmark of effects of, for instance, TCCD and PCBs.

In the slide you see the macroscopy of the thymus of a rat that is exposed to TCDD and you see there is very little left of the normal thymus. Compounds like TCDD and PCBs act through the arylhydrocarbon receptor (Ah), which receptor is very abundant in the thymus. In the next slide you see a cross section of a guinea pig thymus after exposure to PCB and you can see that the cortex is really depleted with respect to thymocytes.

In the thymus, of course, the maturation of T cells take place. So, precursor cells enter from the subcapsular area into the cortex, and a whole maturation process will take place, different receptors are put on and off the precursor T cells to ultimately form the mature T cells, CD4 or CD8 T cells with a complete T cell receptor antigen receptor expressed by them. And after exposure to many chemicals or at least to PCBs and TCDD, this process is frustrated to a certain extent, so the process does not give the same pattern: actually you see relatively more CD8 positive cells and less CD4 cells.

This is another diagram of the thymus, more or less saying the same thing that precursor T cells enter the thymus and this is the maturation process, ultimately the T cells leave the thymus and go into circulation, but in the development of this process, selection takes place in which cells that develop a specificity that is not desired are negatively selected and die by a process of apoptosis whereas the other cells that have desired specificities will be positively selected.

This process is mediated among others by epithelium cells in the thymus. And these epithelial cells are, I would say, a very important target of TCDD and PCBs. In animals that are exposed these epithelial cells are driven into a terminal differentiation to form keratin and losing their function in supporting the maturation process of the thymocytes.

The density of the Ah receptors on these epithelial cells is rather high, and epithelial cells are therefore target of these compounds. In addition, also the thymocyte themselves express these Ah receptors, and can interact with TCDD and PCBs. Therefore, also the thymocyte themselves are the most likely targets of these compounds.

TCDD and congeners such as PCB's, especially planary congeners, that interact with the Ah receptor can affect cellular immunity because of these effects on the thymus that we see. These functional effects can be evidenced, for instance, in the laboratory animals as increase to susceptibility to infectious diseases. When a laboratory animal is exposed to an experimental infection, after also being exposed to PCB, TCDD, decreased resistance is easily found.

One thing that needs to be mentioned about the effects on the immune system of PCBs, TCDD is that usually, the effects are more pronounced if there is a prenatal exposure which is probably a reflection of the fact that thymus is so much involved in mediating the immune suppressive effect. Obviously, the thymus is relevant or active, most pronouncedly at the early stages of life. Adults, both rodents and humans, still have a rudimentary thymus and it is still active to a certain extent but not to the same extent as it is active earlier in life.

Of course it is impossible to intentionally expose people to dioxins and to then investigate effects on the thymus. A way around to do this, is to take fetal thymus tissue, and transplant that together with fetal liver tissue, as a source of lymphocyte precursor cells, under the kidney capsule of SCID mice. SCID mice are severe combined immuno-deficient mice; they do not reject xeno-transplants, so they do not reject human thymus tissue. And actually what you see, after a couple of weeks, is that under this kidney capsule, thymus tissue grows which really resembles normal thymus tissue very well.

Very clear cortex in the medulla demarcation and also the thymocyte distribution are seen in such constructs of the transplanted tissue, that are very similar to a regular human thymus. Fetal thymus tissue is taken after abortion; these adult thymus tissues are taken after an accidental death. You can see that the distribution of CD4/CD8 double positives, or the double negatives, or single positives is very similar in the transplant compared to human situations. Now this construct is easily exposed to different concentrations of your test compounds, and hence effects on the human thymus tissue can easily be studied.

In fact, similar to human thymus tissue, also rat thymus tissue can be transplanted and grow under the kidney capsule of SCID mice. Just to show you that if you do that, you get rat thymus tissue under the kidney capsule of SCID mice which is very similar to the rat thymus in a normal rat. The reason to do that is to be able to compare effects that you would see in the rats with effects you would see in humans, as effects on an intact thymus may obviously differ from effects on a transplanted thymes.

Effects of exposure are seen on the next slide. This is normal grafted human tissue in SCID mice again, and this is the grafted tissue after TCDD exposure. And you can see that, here too, the cortex has been minimalized. Just to compare effects of TCDD on rat grafts, you also see a decrease. The variability is a little bit high, so there was no statistical significance, but I think that the tendency is similar very much to what you would see in a normal intact rat. And in the next slide you see the data expressed as relative cortex to medulla ratio, the effects you would see in human tissue in the SCID mouse as opposed to what you would see in the rat thymus, in a normal intact rat. With this type of information extrapolation of what you would see in intact rats to humans can be done. This is done by comparing what you would see in the rat, the SCID grafted with thymus from the rat, and the grafted human thymus in the SCID, to provide information for the human situation. What you see is that the effects are very similar, exposure wise; indicating that the rat and the human thymus, perhaps the rat and the human immune system, are more or less equally sensitive to TCDD and PCBs. So that is the conclusion of this type of work.

Epidemiological studies have been performed by a group researchers in our country looking for associations between effects on the immune system and exposure to PCBs. They looked in children at the association of cord blood PCBs and the effects on vaccination to mumps, measles and rubella. They found negative correlations actually with all these vaccination studies, but this was only statistically significant in the case of measles. So they found depressed vaccination titres to measles vaccination correlated with exposure to PCBs in cord blood. I should add that measles is very much a T-Cell dependant vaccine. So the antibody responses you get are surely B cell/plasma cell dependent responses, but they are very much governed by T-Cell functions, by helper activity of T-Cells. This is in keeping with the idea that PCBs and TCDD have suppressive action on T-cellular immune responsiveness, even if, as is the case here, the read out is a humoral response. Even if effects on vaccination are observed, this does not imply that protection was hampered. In fact, vaccination is done so that certain variability will not impact protection. However, effects on vaccination titres do signify a functional effect, and such effects may have an influence on resistance to pathogens for which there was no vaccination. The same group of investigators also looked at different pathological phenomena and found that especially with otitis media, middle ear infection, there was an association of higher exposure to PCBs measured over 24 months, with increased incidences of

otitis media indicating that the effect seen on effects on vaccination responses were relevant in terms of resistance, in this case to middle ear infection.

So significant effects on the vaccination responses indicate that the functionality of the immune system was suppressed in such a way that actually resistance to infections, such as here, middle ear infections was depressed, associated with the higher exposure levels. Of course the latter study is an epidemiological study, there is no causality proven, but taken together with the earlier findings these studies indicate that humans encounter effects on immune functions of exposure to background levels of PCBs, which is an important finding.

Laboratory studies usually are able to show causal relationship, the controls of variables is very high, but of course, you need to extrapolate the data to the human situation which is always difficult to do and the relevance may not always be there. Epidemiological studies are obviously very relevant; it is the human population itself that you are studying. But because the control of variables is virtually lacking, or at least very difficult, the evidence for causal relationship is not so strong. Of course, when you have both laboratory studies and epidemiological studies, and when they are pointing in the same direction, things may get stronger.

An intermediate type of studies are formed by semi field studies, where you have a certain control of the variables, and where you may have some relevance, again maybe not to the same extent as in the human population itself, but may really fill the gap between these two more or less extremes of the study spectrum. Such a semi-field study was done in our country in harbor seals. We studied two groups of seals, that we put in basins in the north of our country and we fed them with herring for a period of two and a half years from either the Baltic Sea or the Atlantic Ocean. The herring caught from the Baltic Sea was much more polluted with organochlorines compared to the herring from the Atlantic Ocean. The seals were caught on the coast of Scotland; they had relatively low exposures as they were relatively clean. The reason for this study was a mass mortality among the seals in the Wadden Sea, in the northern part of our country and along the coast of Germany and Denmark, because of a distemper virus, phocid distemper virus, that killed almost half of the population at that time. After that time, the population recovered, and it is very unfortunate that I have to tell you at this moment, again such a disaster is going on. But anyway, what was thought back in the mid 80s, that the severity of this virus epidemic in these animals had to do, at least in part, with chemical pollution in the Wadden sea of where these animals lived and so they would eat fish that was contaminated and would have perhaps a depressed immune system that would then help to bring out the severity of the virus.

I see that my time is running very fast, so I will speed up a little. The next slide shows you exposure levels of the herring that was used, and levels that were ultimately found in the fat tissue of the seals. As you can see on the next slides there were many immune parameters suppressed in these animals. For instance, delayed-type hypersensitivity to sensitisation with ovalbumin, a T-cell dependent response, was suppressed in the Baltic herring group as opposed to the Atlantic herring group. Also antibody production to ovalbumin, which is also a T cell dependant phenomenon, was very much suppressed. Mitogen responsiveness to ConA, pokeweed mitogen PAH was usually significantly suppressed in the Baltic as opposed to the Atlantic group but not the B cell mitogen LPS. As for vaccination responses: the cellular lymphoproliferative response after vaccination with rabies vaccine and tetanus toxoid vaccine were suppressed, but antibody responses to the vaccines were not. Natural killer activity was also suppressed very much in the Baltic as opposed to the Atlantic herring fed seals. So this indicated that the seals fed with Baltic herring showed depressed immune functionality. It does not say of course that their resistance was suppressed. We were not allowed to infect these animals with experimental infections. So what we did was to freeze-dry the herring, or prepare fish oil from the herrings and expose PVG rats, either young adult rats or rats during the perinatal period to freeze-dried fish or fish oil at a level so that it mimics the

exposure that the seals would get and we would then also infect those animals with Rat cytomegalovirus (RCMV).

Then, we found actually decreased resistance in the Baltic herring group of rats; as evidenced by a difference in microbiological burden if they were experimentally infected. Actually you can see that regular food in the rats also has an effect, which puts the risk that we are talking about here a little bit into perspective.

The conclusion of my talk is that organochlorine, including PCBs caused immunosuppression and increased susceptibility to infectious diseases in human and wildlife population. I think that with the three levels of evidence that you have, the experimental levels in the experimental animals, the semi-field study in the seals and the epidemiological studies that has been carried out on humans, we can safely make this conclusion.

In part, these effects are due to effects on the thymus. I guess effects on thymus epithelium cells on thymocyte directly, but there is also effects on B cells, usually these are at somewhat higher concentrations but these have been described that do not involve the thymus.

Perhaps there are other effects, let us say secondary effects through interaction with the neuro-endocrine system as we heard earlier from Prof. Besedovsky that may have these influences. I think it is fair to say that especially the developing immune system is sensitive to effects of exposure and the effects that can be seen can be noted at exposure levels that are encountered in our environment. Thank you very much.

Q&A

Nohara: Thank you very much Dr. Van Loveren. We are going to take a question from the floor.

Matsuzaki: Those are very interesting results; I am very impressed. As the developing immune system is extremely susceptible to chemical substances such as PCBs, what you might call xenobiotics. However, what do you think of this idea? Our ecosystem consists of the food chain, and the immune system we have acquired is made and maintained in the chain of the ecosystem. Rather than the effect of chemical substances working directly on seals such as you indicated, the tiny organisms and microorganisms of the ecosystem are affected first of all. While we ingest this tiny food chain, do you think the immune system that we ourselves have acquired is being destroyed little by little?

Van Loveren: I am not sure I followed the whole question, but I guess what you are saying is that the whole food chain is affected by such chemical that ultimately end up in the seals for instance because they are the top of the food chain. That is what I understood you were saying. And obviously, that is absolutely right.

What has been shown here was the direct interaction of the chemical with immune system as such of the animals, of the seals and in that case, the food chain, of course, did not matter because they were fed artificially with the herring from the Baltic sea or from the Atlantic Ocean. But besides that, I think I agree with you that there is concern of interacting with the food chain will actually damage the animals that are at the top of the food chain. I believe humans are on the top of the food chain as well. Is that a more or less correct answer to your question or?

Matsuzaki: Yes, I understand what you wanted to say about your experiment. O.K. I want to discuss this later with you. Thank you.

Suzuki: Right now that question was very philosophical, but my question is substantial. So the human measles virus and the Baltic Seals infecting virus and this virus, how similar are these viruses as seen in its infectivity or some immune response or induction or so?

Van Loveren: I am not a virologist so I am not qualified to answer this precisely, but as far as I understood, they belonged to the same family of viruses, they invoke similar responses in the seals.

Suzuki: Any particular characteristic that is common in the sea species of viruses?

Van Loveren: It is very much a T-Cell cytolytic T-Cell inducing virus. That is true both for the measles and the distemper virus and the quality of the immune responses were very similar and they belong in the same class, but as I said, I am not a virologist so I dare not to say more.

Suzuki: Thank You.

Nohara: Please.

Lang: Jim Lang, BBL Sciences. You mentioned there has been another distemper outbreak?

Van Loveren: Yes. It is on going right now.

Lang: Are you looking into what chemicals or other agents that may be associated with this particular outbreak?

Van Loveren: Obviously I think we should be aware that even with a normal functioning immune system in a population that has no herd immunity, you can have an epidemic of a virus and that can kill quite a lot of the animals. The organochlorine contamination of the Wadden sea is still there, although it has decreased since the mid 80s, it is not to the same extent. So far there

have been no attempts to study these seals now and really to look into that. The outbreak has been there for about three months. It started in the summer.

Nohara: O.K. Thank you very much, Dr. Van Loveren.