Immunological Analysis of the Low Dosage Chemical Exposure Syndromes

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Thank you for the introduction. I'd like to express my appreciation to our organizer and everyone involved for providing me with the opportunity to be with you here today. Today I would like to speak in clinical terms about the immunological aspects of so-called low dosage chemical exposure syndromes. I will be speaking from the clinician's point of view.

First let me give you a little background information on low dosage chemical exposure syndrome, or LDES. LDES naturally involves a medical definition and various other elements such as philosophical and political elements. When talking about the health of people exposed to extremely low doses of chemical substances, one must be careful not to be offensive. I will speak somewhat about immunity while explaining what LDES is and what causes it, but will not talk about actual clinical symptoms or how patients are diagnosed or cared for.

There are various books written on LDES in the United States and other such countries. LDES is mentioned in Professor Guillette's book in reference to endocrine disruption. Various books on LDES have been published in Japan, but unfortunately volumes that provide systematic knowledge for specialists have yet to be published.

So now let's talk about exactly what LDES is. The syndrome is variously expressed according to the medical point of view, including both the clinical and basic medical point of view, the immunological or perhaps epidemiological point of view or from the administrative point of view, whichever best fits each person's own field.

Most general expression today here in Japan is chemical sensitivity. You may have heard this term from various directions. Related to this is "Sick Building Syndrome," also known as "Sick House Syndrome" in Japan. Those terms apply particularly to the illnesses and syndromes related to indoor air pollution. In foreign countries, particularly the USA, for example, Iris Bell of the University of Arizona use the term "Chemical Intolerance." Chemical Hypersensitivity and Multiple Chemical Sensitivity Syndrome, or MCSS, are used for phenomena that cannot be explained as low dose stimulation syndrome, or mass acute toxicity.

However, people such as Professor Kilburn of the University of Southern California view low dosage chemical exposure syndrome as so called "Chemical Brain Injury." In relation to low dosage chemical exposure syndrome, this term expresses the condition whereby the central nervous system, primarily the brain, is damaged by chemical substances. This is the view of chemical exposure from the neurological standpoint. From the viewpoint of an immunologist, this is caused by chronic activation of the immune system. Therefore this is called "Chronic Immune System Activation." It also may be referred to as "Total Allergy Syndrome."

At any rate, various terminology is used by experts for various types of research currently being conducted on such illnesses depending on their viewpoint. Today I would like to use the term "Chemical Sensitivity," the most commonly used expression here in Japan.

In Japanese, many terms are used to describe illness such as disease, disorder, illness and syndrome, but none apply to chemical sensitivity. Or perhaps they all apply. So it is difficult to make it fit these terms at the present stage. In this form, we currently use the term "syndrome" because it contains various symptoms

Back to the beginning of this presentation, we will talk about what chemical sensitivity is. This was defined in the "Archives of Environmental Health" in the 1999 consensus for multiple chemical sensitivity. In short, chemical sensitivity can be described as a chronic condition that occurs from low dosage chemical exposure by which people are generally not affected.

Characteristic of many chemical sensitivity patients is a reaction to various structurally different unrelated chemicals, more specifically, to wide range of diverse chemicals that do not have common chemical structure. This is defined as multiple chemical sensitivity, or MCS particularly adding the word "multiple."

In order to establish chemical sensitivity, a 2-step mechanism is currently considered. This was proposed by Ashford of MIT in Boston and Miller et al of the University of Texas at San Antonio in 1998. The two steps are initiation and elicitation. In other words, comparatively large exposure at first, or relatively unbroken low dose exposure over a period of time such as exposure to volatile organic compounds, pesticides and insecticides containing EDs. This causes subsequent manifestation of various clinical symptoms to appear at minute, usually nontoxic level exposure. We currently understand that this is a 2-step process.

One of the ways our bodies are affected by low dosage chemical exposure to so-called xenobiotic environmental chemicals is through the nervous system. Another is through the immune system, which is today's theme. It is difficult to translate the term "sensitization," into Japanese. All you have to know is a plasticity occurs in the nerves, and the nervous system and immune system are sensitive to those chemicals. There is crosstalk between the immune system and the nervous system, so this is perhaps a model by which chemical sensitivity will be established. From the perspective of the nervous system, this is chemical brain injury, and you can see that this is chronic immune system activation from the perspective of the immune system.

It is easy to link high-dose acute toxicity with clinical symptoms, but it is difficult to link low dosage chemical exposure. What makes it difficult is the fact that some people exhibit symptoms, in other words, some are affected, while others are not. One of the factors behind this is genetic detoxification ability. However, biological, physiological and psychosocial factors of each people are also very important when the body is affected by a certain substance.

In other words, when the body is affected by chemical substances, there is a possibility that the person may already have a basic illness such as diabetes or hypertension. Biological factors such as whether the person suffers from extremely strong atopic dermatitis or bronchial asthma must be given a lot of weight when considering the degree of effect of chemical substances. Other physical stimulation such as high or low temperature, high or low air pressure is also a related factor. From a clinical standpoint, we know that psychosocial factors, particularly stress, have an extreme effect on clinical symptoms while establishing the cause of such diseases.

When looking at the effects of exposure to chemical substances in the environment just from the perspective of immunization, we currently have a lot of information. One is the relationship with allergies. In some cases an allergy is caused by chemical substances or the symptoms of the allergy such as atopic dermatitis and bronchial asthma are exacerbated by chemicals in the environment. Another important thing is the relationship with autoimmune disorder. We have conducted basic research energetically in this field since about 1996 or 1997 on substances including endocrine disrupters. EDs clearly orient the human immune system toward autoimmunity. This slide shows various other immunological phenomena. In other words, we currently have come to know quite a bit about the effect of such chemicals existing in the environment on the immune system and various diseases of the immune system.

Although somewhat basic, here are our research reports up to now, and these are the results of animal experiments involving the effect of environmental chemicals on the immune system rather than chemical sensitivity we talked about today. At least one thing we learned from animal experiments including some human experiments is that xenobiotics (non biological materials/foreign substances) including EDs affect the thymus, in other words especially differentiation and maturity of T cells of the thymus as the primary immune organ. This will probably come up again when we talk about dioxin.

We have also learned that xenobiotics also have an extreme effect on various subsets such as peripheral T lymphocytes. Animal experiments have shown that they also effect the B lymphocyte system as well. It is quite difficult to learn this from human beings, but the effect is seen quite a bit in animal experiments.

More importantly is the linkage with autoimmune disorder. Such environmental chemicals alter immunity so that it becomes autoimmune disorder. By now we know it causes stronger autoimmunity.

Since 1994 we have conducted various experiments concerning sex steroids and immunity. These are the four projects on which we have devoted most of our attention when studying the linkage between EDs and sex steroids.

For one thing, we reported in 1998 that so-called estrogenic xenobiotics having an estrogen effect affects the signal cascade of mitogen response in human peripheral lymphocytes.

Another thing is thymic epithelial cells which take charge of various types of education to immature T lymphocytes in the thymus. We have already reported that estrogen imitators in the environment inhibit secretion of hormones from thymic epithelial cells.

We presented the results of recent animal experiments at the meeting of the Society of Immunotoxicology held last September. If you examine the intact thymus of a rat with a scanning electron microscope (SEM), you can see that the lymphocytes are packed tightly together like this. When administered dibutylpthalate (DBP), which is a type of phthalate ester, the number of lymphocytes decreases causing the thymus to become less dense, apoptosis is induced to an extreme degree, and apoptosis such as can be seen in bleb is observed.

At the annual meeting of Japan Society of Endocrine Disrupters Research held in parallel with this symposium, Dr. Kawakami of Kitasato University has reported that the thymus is part of the immune system which is related to the enteric canal, the secondary immune organ. This is Genistein, but if administered for a comparatively long period of time, apoptosis is significantly induced in this area. If seen through a transmission electron microscope (TEM), animal experiments have shown that apoptosis is extremely induced in the immune system when it is eaten by macrophage.

Now I would like to talk a little about how the immune systems of patient with LDES in particular are disrupted. From the clinical point of view, we could perform detailed analysis of immunity and subsets when using animals. In the case of human beings, due to the limitation of medical treatment, the elements we could study were naturally limited because we studied various things through patients. There are many problems such as the problem of health insurance – it would be ideal if we could conduct the same detailed analysis of a basic subset with human beings as with animal experiments, but in reality we cannot.

So today I would like to show you how patients are affected by low dose exposure at least by means of the ratio of CD4/CD8, the percentage of lymphocytes and a DNA histogram of peripheral lymphocytes.

These are representative patients; the sex and age of each patient are given here. The causes of sickness in these patients are various, such as formaldehyde or toluene accompanying so-called

“indoor air pollution.” These illnesses include the aroma of plasticizer such as phthalate ester in the air.

As for low dose exposure patients, there is an extremely limited amount of data on immune system such as white blood cell count and lymphocyte percentage, or CD4/CD8 ratio. As far as I have seen, of course some of these patients are normal, the CD4/CD8 ratio is extremely high. From the overall standpoint, in most of LDES patients CD4/CD8 ratio is relatively high and they often have an extremely low total number of lymphocytes.

Here is an example of two patients; Japanese is given on this side. This is “low” and this is “high.” Here is CD4+ and this is a double negative. This is CD8+ and this is a double positive. Then there is the CD4/CD8 ratio. As you see in this case CD4+ is high, CD8+ is low, and consequently the CD4/CD8 ratio is high. In this case, CD4+ is within the range of standards, but because CD8 is suppressed to an extreme degree, CD4/CD8 ratio is high. Many LDES patients exhibit this tendency.

These are the actual results. In all cases CD4+ is high, CD8+ is low and the ratio is high. The immune profile for autoimmunity is frequently observed.

These figures in the first abstract and the reexamined portion differ somewhat in some places, but are basically the same. These figures are the results of 221 LDES patients that came to the Kitasato Institute.

These are statistics from the environmental medical center called Environmental Health Center which is located in Dallas, Texas. As the difference in number of cases and difference in equipment used for measurement between Japan and the USA has resulted in various problems concerning sensitivity and so on, the figures differ somewhat. In any case, CD8+ is often low. Consequently CD4+ is high. When combined with this, the CD4/CD8 ratio is high: even in the USA it’s a little more than 1 to 5.

As a percentage, it doesn’t mean all of these diseases cause immunity abnormalities, but if you look at normal people, generally such low-high is caused in about 5%, or at most 6% or less. This means that immunity is clearly unbalanced in LDES patients.

As for another one, the percentage is higher than given in the abstract because we have reexamined it more closely. If you take a DNA histogram of the peripheral lymphocytes, 1 peak of most ordinary people, so-called “DNA content of 2N people,” appears. Here appears another peak for such LDES patients. Besides the so-called “2N peak,” an aneuploid peak appears. Although there are varying degrees, this aneuploidy appears in almost 20% of all patients. This is very interesting. If you just look at this, the findings closely resemble phenomena like leukemia. What is different is they depart from such exposure. In other words, as patients’ condition improves when they depart from exposure, we observe the phenomenon where this returns to normal, as it was before. DNA histograms for peripheral lymphocytes are therefore extremely effective for following up low dosage chemical exposure syndrome in such patients.

Such disruption of the immune system is naturally relevant to the nervous system. A little while ago we heard how cytokines produced from the immune system and made by the nervous system itself. From this standpoint, sensitization and sensitivity of the immune system to chemical substances nonetheless affect the patient’s behavior, especially general activity, social and sexual behavior, ingestion of food/appetite and drink. Brain self-stimulation, body care, learning and memory ability decrease markedly. A condition of so-called “chronic fatigue” occurs, whereby the patient becomes extremely fatigued, and has no will to do anything.

For example, one of the questions is how the effect of chemical substances is transmitted as a signal. One thing is exogenous environmental factors are related to response of the immune system. Another is these naturally affect the endocrine system. Yet another is they affect the nervous system.

There is crosstalk between the immune system and nervous system. This results in impairment of autonomic nervous system regulation. Along with symptoms of the nervous system such as impairment of emotions, cognition and coordination, the immune system does not behave normally. In this way, I think you will be able to understand the relation if you look at them one by one.

To be more specific, such environmental chemicals disrupt the immune system. Cytokine from the immune system affects the central nervous system through centripetal nerves. Various types of cytokine are produced here also. If this acts to region such as the hippocampus of the limbic system, it can lead to altered behavior. If this goes to the hypothalamus, it will disrupt the endocrine system as well as produce fever. If it affects the spinal cord, it will affect pain and so forth. Such simple disruption of the immune system therefore ultimately affects the human nervous system and endocrine system. This has one important cascade.

I will introduce literatures that cover this in basic terms. One is “Toxicant-induced Loss of Tolerance” by Claudia S. Miller and the other is “Neural sensitization model for multiple chemical sensitivity: overview of theory and empirical evidence” by Iris R. Bell of the University of Arizona that covers in particular chemical sensitivity concerning neural sensitization. These two literatures provide a good understanding of LDES.

To summarize this, in conclusion we can say the CD8+ level is low for LDES and the ratio consequently rises. Unfortunately our observations were clinical observations only, so a future theme for clinical research is to analyze the detail as to why this occurs.

I would like to express my appreciation to cooperating researchers Professors Ishikawa, Miyata and Aizawa of Kitasato University, Professor Yoshida of Asahikawa Medical College, Professor Kayama of Jichi Medical School, Professor Aikawa of Tokai University, Iris Bell of the University of Arizona, Claudia Miller of Texas and William Rea of Dallas. Last but not least I would like to thank Ana M. Soto and Carlos Sonnenschein of Tufts University, who were my instructors in the USA.

Q&A

Nohara: Thank you very much. We shall now move on to a discussion. Do we have any questions from the floor?

Q: I believe that in some of your patients you mentioned that they were suffering from chemical sensitivity due to indoor factors like formaldehyde. How did the patients express the sensitivity and how can you be sure how to do with the formaldehyde?

Sakabe: We live exposed to complex contamination. It is difficult to say whether a person's clinical symptoms are due to formaldehyde only, or from toluene only or phthalate ester only for that matter. We do however have the technical capabilities to measure what constituents of room air contamination are high and which are low. So when a patient comes to us, we can check out which substances in his living environment are high and which are low. We are currently checking to see which clinical symptoms appear by conducting a patient load test for various chemical substances with priority on high substances.

Nohara: I have a question. You said concerning the balance of CD4/CD8 pertaining to chemical sensitivity of patients, CD4 was high. Is this a result or a cause? Does this mean people with high CD4 tend to be more sensitive or tend to become sensitive? Or does CD4 become high when a sensitive person is exposed to chemicals?

Sakabe: In conclusion, I would say both. Naturally the mechanism by which chemical substances act on the immune system differs according to the substance. If you consider only endocrine disrupters for example, there are more female hormone receptors for CD8. CD8 is therefore attacked by such receptors and is affected. I think therefore the immune balance relatively favors CD4.

Then people with this illness tend to have atopic dermatitis, bronchial asthma and some sort of illness of the immune system beginning from when they are children. Although it may not be a genetic cause, these people will tend to have poor balance of immunity, and if environmental factors are added, the symptoms will become worse.