Malignant Histiocytosis in the Bernese Mountain Dog: Study of the physiopathology and genetic causes

Written from notes by Pat Long from the presentation by Dr. André.

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In the study of the genetic basis for disease, it must first be determined if the disease is probably genetic. Pedigree study is used for this. The aim of canine genetic research is to develop genetic tests, and to determine if it is a homologous disease in humans – in other words, does it involve the same genetic mutation in humans.

Canine histiocytic poliferative disorders are hetergenous diseases that include reactive disorders such as cutaneous and systemic histiocytosis, and neoplasia such as cutaneous histiocytoma and localized and disseminated histiocytic sarcoma (malignant histiocytosis). It involves the accumulation and proliferation of histiocytes in specific tissues.

1. Cutaneous histiocytoma:
   Cutaneous histiocytoma is the proliferation of epidermal langerhans cells. It is found more in young dogs, there is no breed disposition, and it normally regresses.
2. Reactive histiocytic disorders:
   Cutaneous histiocytosis is characterized with cutaneous lesions. It is called systemic histiocytosis when extensions to the lymph nodes or other tissus is observed. It is familial in Berners, and is generally seen in young adults aged 4 to 5 years. It may be an immune system deregulation.
3. Malignant histiocytosis (Histiocytic sarcoma):
   Malignant histiocytosis is an aggressive disseminated histiocytic sarcoma, found in the lungs, spleen, liver, bone marrow, among other organs. There is a high incidence of the disease in BMDs, and it represents nearly 25% of the cause of death in the breed, perhaps more – it is probably under diagnosed. It was found to be genetic (Padgett et al. 1995). About 80% of all cases are found in Bernese, Rotties, Goldens, and Flat Coated Retrievers.

The clinical signs of MH can involve: loss of weight, reduction of appetite, or a bad mood. Tumors in the lung may be indicated by a cough, and tumors

in the liver may be indicated by pale gums. It may be found in one organ, or it may be multifocal.

The diagnosis can only be done by cytology and histology. Immunohistochemistry is needed in borderline cases. Dr. Peter Moore at UC Davis has developed special reagents to assist with this.

Histiocytosis shares many clinical and pathological features with Langerhans cell histiocytosis in humans, for which the underlying causes are still unknown. It occurs in children typically under the age of 5 years, and it is rare. There are only about 50 cases per year in France. It can evolve to dramatic tumorgenic form, which resemble canine MH.

MH in Berners is a unique spontaneous model for human genetic study, so it is ideal for genetic research. The goal of the research is to develop genetic tests, aid in diagnosis, and ultimately to prevent the disease in canines and to facilitate the search for the genes responsible of the human forms of Histiocytosis.

The search of the genetic bases of MH in the BMD has been undertaken:

- In France, at the CNRS of Rennes (Lab of Catherine André), a family of more than 300 dogs has been collected, 103 of those dogs were affected. All affected dogs in the study have a histopathological report and are submitted for histological confirmation and characterization. There were 49 males, 54 females. There were 33 unaffected dogs all over the age of 10 years.

  A segregation analysis is underway to determine the mode of transmission. Up to now, a transmission mode involving a small number of genes has been hypothesized (oligogenic transmission). Moreover, owing to a questionnaire completed by the veterinarians who are referring the MH cases, clinicopathological data are under investigation. A better characterization of the condition is also underway through immunohistochemical analysis. The genetic analysis is undertaken by a so called "Genetic Linkage Analysis" on 200 dogs of the family and by the collection of tumor tissues to analyze gene expression at the RNA level.

- In the US, at the NIH, Bethesda (Lab of Dr. Elaine Ostrander), a set of 100 cases and controls from American Berners are also being collected, and genetic analyses are underway. This is called an "association study" using a population of unrelated healthy and affected dogs. 60 affected and 125 unaffected BMDs were analyzed.

- In the US, at the North Carolina University, in Raleigh (Lab of Dr. Matthew Breen) another genetic study is ongoing on fresh tumor tissue samples to find chromosomal abnormalities.

These 3 genetic studies aim to identify specific genes that are involved in the disease.

In France, at the CNRS of Rennes and the Veterinary school of Nantes (C. André and J. Abadie), clinicopathological data were obtained from the analysis of 60 affected dogs. The data for this study will be published in 2007, and updated here.

There is a high prevalence of MH in Berners in France. Incidence is similar in males and females. The mean age is 6 years. There is no association with other proliferative histiocytic diseases, and no association with vaccines, infectious diseases, or environment. There is a very homogeneous clinical presentation, and a poor prognosis.

After 10 years of mapping the canine genome, 10,000 genes were finally mapped (Hitte et al., 2005 : a collaboration between C. André and F. Galibert – France, E. Ostrander –US, M. Breen –US, E. Kirkness –US, and P. Deloukas – GB) and the sequencing of the entire genome was completed last year (Lindblad-Toh et al., 2005, international collaboration). 2,000,000 polymorphic markers, or SNPs were identified. Among the 2000 microsatellite markers, 300 microsatellite markers well distributed throughout all the chromosomes have been selected for genetic studies.

Now these tools are ready to serve for genetic mapping with complementary methods in the study of Dr. C. André and E. Ostrander, in collaboration. Both studies use the same sets of markers but different samples (a family in France, a series of non related cases and controls in the US). Experiments and statistical analyses are being done. In parallel, tumor samples have been collected to analyze RNA and gene expression of candidate genes (among others growth factors, receptors, adhesion molecules, interleukins…).

If you want to participate in the study, please see the protocol by clicking here.

Due to shipping times, all European samples should be sent to Dr. André's lab in France, and all North American samples should be shipped to the researchers Dr. E. Ostrander or M. Breen in the USA.

The following would be needed:

- Pedigree
- Blood sample in an EDTA tube
- Knowledge of whether the dog is affected or not
- Histopathological report
- Tumor and healthy tissue (fresh, -20°C frozen or in paraffin)