RESEARCH PROGRESS REPORT SUMMARY

Grant 01771: Defining the Unique Genetic Markers in Dogs That Define Immune Function, Disease Resistance and Tissue Transplantation

Principal Investigator: Dr. Beverly Torok-Storb, PhD

Research Institution: Fred Hutchinson Cancer Research Center

Grant Amount: $178,200.00

Start Date: 1/1/2013 **End Date:** 6/30/2017

Progress Report: End-Year 4

Report Due: 12/31/2016 **Report Received:** 12/27/16

Recommended for Approval: Approved

(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)

Original Project Description:

The Major Histocompatibility Complex (MHC) is a region of the genome that contains genes that code for a group of cell surface proteins known as Dog leukocyte antigens (DLA). DLA play important roles in the immune system including the recognition of self as well as recognition of foreign pathogens such as bacteria and viruses. The MHC genes are highly polymorphic and each gene has many different forms or alleles. Matching of MHC alleles between the donor and recipient is important for the success of stem cell and tissue transplants. Specific assortments of MHC alleles or haplotypes have been associated with an increased risk for the development of diabetes and auto immune diseases in humans. Knowledge of these associations has been valuable in understanding disease mechanisms. Recently we have developed improved methods for identifying the different forms of the DLA genes, in a large number of dogs of diverse breeds. In this application, we propose to characterize haplotypes, in over 1200 dogs from at least 50 breeds using a high throughput sequencing strategy. The distribution and frequency of different forms of each of these genes and their specific clustering among different breeds will greatly enhance our knowledge of the genetic diversity among breeds. The methodology and data gained from this study will enhance the power of association studies between MHC types and canine diseases. Such a database will also enable tissue transplantation from unrelated but matched donors as a treatment for advanced malignancies and other diseases, among dogs of most breeds.

Grant Objectives:

The goal of the project is to construct haplotypes of DLA alleles from about 1200 dogs of about 50 AKC pure breeds. The methodology and data gained from this study will identify the level of diversity of DLA alleles in different breeds and enhance the power of association studies between DLA haplotypes and canine diseases. Such a database will also enable tissue transplantation from unrelated but matched donors as a treatment for advanced malignancies and other diseases, among dogs of most breeds.

Publications:

- Venkataraman GM, Geraghty D, Fox J, Graves SS, Zellmer E, Storer BE, Torok-Storb BJ, Storb R. Canine DLA-79 gene: an improved typing method, identification of new alleles and its role in graft rejection and graft-versus-host disease. Tissue Antigens. 2013 Apr; 81(4):204-11. PubMed PMID: 23510416; PubMed Central PMCID: PMC3605710.

- Tsai KL, Starr-Moss AN, Venkataraman GM, Robinson C, Kennedy LJ, Steiner JM, Clark LA. Alleles of the major histocompatibility complex play a role in the pathogenesis of pancreatic acinar atrophy in dogs. Immunogenetics. 2013 Jul; 65(7):501-9. Epub 2013 Apr 21. PubMed PMID: 23604463.

Report to Grant Sponsor from Investigator:

The Major Histocompatibility Complex (MHC) is a region of DNA on a chromosome that encodes genes that help control the immune response. The proteins encoded by the genes of the MHC are displayed on the surface of white blood cells (leukocytes) and are referred to as dog leukocyte antigens (DLA). DLA provides cells with the ability to distinguish foreign pathogens such as bacteria and viruses from self. The MHC genes are highly polymorphic i.e., each gene has many different forms or alleles. Matching these MHC alleles between the donor and recipient is important for the success of stem cell and organ transplantation.

Specific “patterns” of MHC alleles are inherited on dog Chromosome 12, one from each parent. The unique pattern of alleles within the MHC of one parental chromosome 12 constitutes a haplotype. Since an entire chromosome 12 is passed on from each parent it is easy to perform DLA-typing to match for haplotypes within a family. However matching haplotypes from unrelated dogs is more challenging since different patterns of alleles may be present in their MHC.

In humans, finding an unrelated donor with an MHC match for both haplotypes requires a registry of hundreds of thousands of potential donors, a number predicted by the known diversity of the human MHC. Defining the diversity of the canine MHC will help create strategies for more accurate DLA-typing and thereby improve transplantation results from unrelated donors.

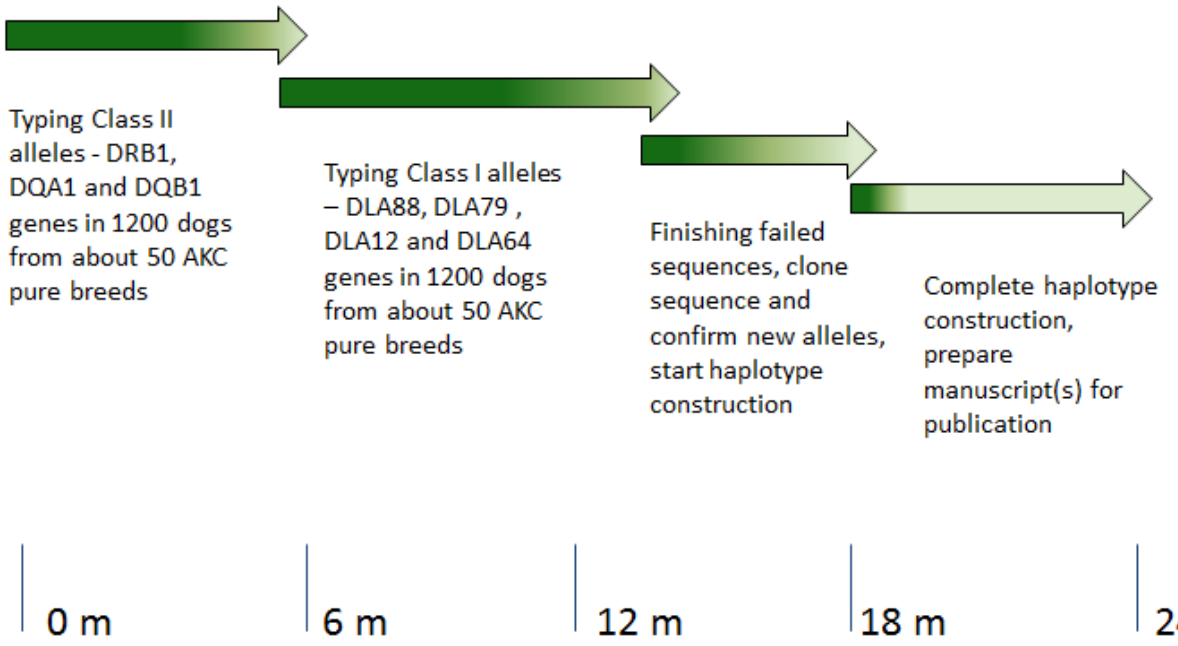
Better matching of MHC genes between prospective donor –recipient pairs is only one important consequence of better DLA-typing. Importantly, since the MHC controls the immune response, a better understanding of the diversity of MHC genes may help us to discover an association between these genes and various diseases, which in turn should inform mechanistic studies to define disease processes and develop new therapeutics.

The goal of the project is to construct haplotypes of DLA alleles from about 1200 dogs of about 50 AKC pure breeds. This can be divided into 4 major tasks.

1. Typing the 4 polymorphic class I genes
2. Typing the 3 polymorphic class II genes
3. Cloning and/or confirming new and ambiguous alleles for all class I and II genes.
4. Analyzing and deriving haplotype compositions.

Aim 1, nearest allele identities have been established for two of the 4 class I genes, viz., DLA88, and DLA79 in over 1000 dogs. An unexpectedly large number of new alleles have been found for both of these genes. This has necessitated more work than anticipated to clone and confirm new alleles, Aim 3. Aim 2, Allele identities have been established for all the three class II genes, viz., DRB-1, DQA-1 and DQB-1 in over 1000 dogs. Many new alleles have been found for all three genes. We are requesting a no cost extension of 6 months towards characterizing the large number of new class I alleles we identified. Aim 4, haplotype compositions will be finalized after allele identification is complete. Initial typing of DLA64 and DLA12 genes are complete. Haplotyping analysis is in progress.

Specific haplotypes have been associated with an increased risk for the development of diabetes and auto immune diseases in humans. Knowledge of these associations has been valuable in understanding disease mechanisms. The distribution and frequency of different forms of each of these genes and their groupings among different breeds will greatly enhance our knowledge of the genetic diversity among breeds. The methodology and data gained from this study will enhance the power of association studies between MHC types and canine diseases. For example, in collaboration with Dr. Leigh Anne Clark's group at the Clemson University in South Carolina we have identified an association of a specific DLA class I allele with pancreatic acinar atrophy in the German Shepherd Dog. Such a database will also immediately enable tissue transplantation from unrelated but matched donors as a treatment for advanced malignancies and other diseases, among the dogs of most breeds.



We have completed the proposed amplification and initial sequencing of all the 7 polymorphic alleles of the 1200 dogs set. However, further detailed characterization is focusing on the two most polymorphic class I (DLA-88 and DLA-79) genes as we observed an unexpectedly high number of new alleles. Alleles of all three class II polymorphic genes, viz., DRB-1, DQA-1 and DQB-1 were typed in the population of dogs as proposed in the grant. Work is being done in batch mode to confirm new alleles for logistics and cost efficiencies. There is no change in the aims or methodology of the experiments, only in the order of executing methodologically independent steps.