THE PARR ASSAY FOR CLONALITY IN THE DIAGNOSIS OF LYMPHOPROLIFERATIVE DISORDERS

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Lymphoproliferative disorders can present with a myriad of clinical signs, reflecting the diverse nature of the cells which give rise to them. Often the diagnosis is straightforward, and can be made by cytologic or histologic evaluation of tissue, bone marrow or blood smears. However cases of early lymphoma when the neoplastic cells are a minority of the total population, and cases which involve small, benign-appearing lymphocytes may require additional diagnostic testing in order to make a definitive diagnosis.

The PARR assay (PCR for antigen receptor rearrangements) was developed to detect clonally expanded lymphocyte populations in dogs 1, and is now also being developed for cats 2, 3. The test is based upon the observation that individual lymphocytes can be uniquely identified by the constellation of antigen receptor genes that they carry – immunoglobulin genes in B cells and T cell receptor genes in T cells.

During development, rearrangement of V, D and J gene segments and somatic hypermutation create genes with antigen-binding regions that are unique in length and sequence. Thus, in a heterogeneous population of lymphocytes, such as in a response to an infectious agent, there will be a diversity of antigen receptor genes, whereas in a neoplastic lymphocyte expansion, the antigen receptor genes will be homogeneous in sequence and length. The nature of the antigen receptor gene is determined by PCR amplification of genes encoding the antigen binding regions, followed by any one of several methods which can distinguish PCR products by size and/or sequence.

The assay as performed in our laboratory detects 80% of confirmed cases of lymphoma and lymphocytic leukemia, and will be falsely positive (i.e. a clonal population of lymphocytes will be identified when there is no neoplastic population present) in 8% of cases. Reasons for false positives include, but are not limited to, a small number of cases of *Ehrlichia canis*, Rocky Mountain Spotted Fever and Lyme disease. We have also found that in rare cases of acute myelogenous leukemia, aberrant antigen receptor rearrangement can be detected, similar to what is found in human medicine.

PARR has a number of uses in the diagnosis and characterization of lymphoproliferative disorders. The most common reason for submission of samples is when there is a suspicion of lymphoid neoplasia that cannot be confirmed by other means. Examples include equivocal cytology and histology, idiopathic hypercalcemia, and lymphocytosis of small mature lymphocytes. For example in a recent study examining ancillary methods for diagnosing mediastinal masses, we found that 7/7 lymphomas, but 0/6 thymomas had clonally rearranged antigen-receptor genes (Lana et al, *in press*), making this test useful for distinguishing these two common mediastinal masses. We have also completed a study of chronic lymphocytic leukemia (CLL), in
which we found that many cases of CLL can present with lymphocyte counts of fewer than 20,000. The finding of a clonal lymphocyte population identifies these cells as leukemic rather than reactive.

Because the sequence of the gene segment encoding the antigen binding region is unique in each lymphocyte, this sequence can be used as a molecular fingerprint of the neoplasm. This fingerprint has been employed in human medicine to make some seminal observations. First, the unique immunoglobulin sequence carried by B cells of a MALT lymphoma in a patient with *Helicobacter pylori* was used to explore the gastric lymphoid hyperplasia that preceded the lymphoma. This study demonstrated the B cells that gave rise to the MALT lymphoma could be found in the hyperplastic lesion 10 years previously. We are currently employing the same methods to determine if lymphoplasmacytic enteritis in cats is a pre-neoplastic state that eventually leads to GI lymphoma.

Molecular fingerprinting using the unique gene sequences of the antigen-binding region was also used to demonstrate that the Reed-Sternberg cells in human Hodgkin’s lymphoma are B cells. This study took advantage of the observation that in some patients, histologically identified non-Hodgkin’s lymphoma, and Hodgkin’s lymphoma can occur simultaneously (as identified by histologic appearance). Using laser capture microdissection, the investigators sequenced immunoglobulin gene segments from individual Reed-Sternberg cells and cells that comprised the B cell lymphoma in the node of two patients. The gene sequences were identical between these two cell types, providing unequivocal evidence that the tumors were derived from the same clone. In dogs, neoplastic with apparently morphologically distinct features can co-exist or appear sequentially in the same patient. We identified an extreme example of this in a patient that first presented with a B cell lymphoma. The lymphoma responded to chemotherapy and went into complete remission. The dog then represented with a tumor that was functionally and cytologically diagnosed as multiple myeloma. Despite the dramatic difference in the appearance and behavior of the tumor cells, both could be unequivocally shown to have come from the same clone because they carried the same immunoglobulin gene sequence.

Another use for the PARR assay is in monitoring minimal residual disease (MRD) after chemotherapy. The treatment of human acute lymphocytic leukemia (ALL) has been significantly affected by the ability to detect minimal residual disease using the unique gene sequences carried by lymphocytes. Once the sequence has been determined, tumor specific primers, which will only amplify DNA from the tumor, are produced. Such tumor specific primers are estimated to be 100 to 1000 times more sensitive than the conventional primers which are made to identify as many antigen receptor genes as possible. When such highly sensitive primers are used to determine if there is low level disease remaining after chemotherapy for ALL, disease outcome was significantly affected by the presence of MRD. We have begun studies to determine if the presence of MRD after chemotherapy in canine lymphoma has prognostic significance. At present, we have detected MRD using the broadly reactive primers used in the conventional PARR assay, but because sequencing is relatively inexpensive, the ability to detect MRD with a high level of sensitivity is well within the means of veterinary oncologists.
Based on our clinical experience with this assay, we suggest the following guidelines for clinical situations in which the PARR test is a useful diagnostic tool, and other situations where it is not useful.

**The PARR assay is useful in**

- Detection of lymphoproliferative disease when cytology or histology is ambiguous
- Determining the clonal relatedness of lymphoid malignancies
- Retrospectively searching for pre-neoplastic lymphocyte expansion
- Detection of minimum residual disease (?)

**The PARR assay is not useful in**

- Immunophenotyping if flow cytometry, immunohistochemistry or immunocytochemistry are available
- Staging lymphoma
- Distinguishing lymphoid from myeloid lineage neoplasms
- As a screening assay in healthy animals.