Immunological receptor rearrangements in B- or T-cells can be tested to provide information on the clonality of the cells within the sample; this can be helpful in the diagnosis of lymphocytic proliferations. In general terms, benign expansions of lymphocytes are usually polyclonal, whereas malignant conditions are most often monoclonal. This article briefly outlines the molecular basis of this type of assay, its clinical utility, and some of its limitations.

Immunological receptor gene rearrangements

Mature B- and T-cells both have immunological receptors on the cell surface. In B-cells, cell surface immunoglobulins (Ig) consist of a heavy chain and a light chain, which are encoded by heavy chain (IGH), and light chain genes: IGK for kappa light chains and IGL for lambda light chains. Only one of the two types of light chain gene is expressed and included in the final Ig receptor in each B-cell. In T-cells, the immunological receptors are dimers, encoded by T-cell receptor (TCR) genes: TCRA and TCRB, or TCRD and TCRG, which produce ‘alpha-beta’ or ‘delta-gamma’ T-cell receptors respectively. The proteins encoded by these genes all have complementarity determining regions (CDRs) whose molecular shape determines which specific antigens they bind. CDRs need to be very diverse, so that the multiple potential antigens in the environment can be detected by at least one B- or T-cell clone. CDRs consist of two or three segments, known as the V, D and J segments. Specific CDRs are produced within each B- or T-cell during maturation, in a process known as V(D)J recombination, or gene rearrangement.

The CDRs of the immunological receptor genes are arranged in a modular fashion (see Figure 1 below for an example, showing the IGH locus). During early lymphoid maturation, V, D and J segments of DNA encoding the final Ig or TCR CDR in each cell are joined together (recombined), with elimination of intervening segments – for example, in Figure 1, VH4 is joined to DH3, and then to JH4 to form the final CDR, which is transcribed and translated into the Ig heavy chain. Selection of the V, D or J segments used in each cell is relatively random, and there are a large number of segments to choose from – e.g. at the IGH locus there are 44 usable V segments, 27 D segments and 6 J segments. This leads to approximately 2x10^6 different possible combinations of VDJ segments.

PCR-based gene rearrangement assays – using diversity to identify clonal proliferations

The diversity of the Ig and TCR V(D)J regions can be exploited to determine whether a B- or T-cell proliferation is clonal. Sets of polymerase chain reaction (PCR) primers have been designed to amplify across the recombined CDR region of the different immunological receptor genes (primarily IGH, IGK, TCRB and TCRG). For each gene, multiple primers are combined in one assay, in order to bind to as many of the ‘V’ and ‘J’ segments as possible and thus amplify most of the possible VDJ combinations. During PCR, the area between the primers is exponentially amplified. Following this, the PCR products are separated by size using electrophoresis, and detected. If a sample contains a polyclonal population of lymphocytes, then there will be a range of different rearranged VDJ segments. The size distribution of PCR products from a polyclonal cell population tends to be near Gaussian, largely because of junctional diversity as described above. This is shown in Figure 2 below.

Conversely, if there is a proliferation of monoclonal cells arising from the same original progenitor, as in a lymphoid malignancy, then the final V(D)J segment from that specific clone will predominate over the background ‘normal’ polyclonal pattern. This is shown in Figure 3 below.

Clinical utility of PCR-based gene rearrangement testing

Determining whether a B- or T-cell proliferation contains a clonal population is particularly useful in lymphomas, where clinical history, histological appearance and immunophenotype may not be sufficient to make a definitive diagnosis.

In skin pathology, cutaneous lymphomas such as mycosis fungoides can sometimes be difficult to differentiate from non-malignant inflammatory dermatoses. Presence of a clonal population by PCR supports the diagnosis of a malignant lymphoproliferation rather than a non-clonal, inflammatory proliferation. Gene rearrangement assays are therefore normally ordered as an additional test by a histopathologist, after they have examined the histological appearance and immunophenotype of the sample.

B-cell proliferations are usually tested by IGH and/or IGK rearrangement PCR; IGH rearrangements are present in all B-cells, while IGK rearrangements occur before IGK during B-cell maturation, and are usually present even in lambda light chain-restricted cells. T-cell proliferations are usually tested by TCRB and/or TCRG rearrangement PCR.

Limitations of PCR-based clonality testing

- False negative results
- PCR primers used in gene rearrangement assays are designed to bind to the majority of possible V and J segments. However, they cannot detect all segments, due to the limited number of primers that can be combined in a single PCR reaction.

In addition, DNA is generally extracted from paraffin-embedded biopsy samples that have been fixed in formalin, which is known to damage DNA. If a biopsy contains a low number of lymphocytes (e.g. a paucicellular infiltrate), or if the quality of the extracted DNA is poor (e.g. due to sample age or over-fixation), sensitivity for detecting clonal populations can be reduced. Therefore, the quality and amount of extracted DNA is assessed before proceeding with gene rearrangement.

Figure 1: The modular arrangement of the V, D and J segments of the IGH gene, and the process of VDJ recombination/rearrangement in a maturing B-cell that produces the final CDR segment.1

CDR diversity is expanded further because the ‘joining together’ process in all of the immunological receptor genes is error-prone – during recombination, random nucleotide bases are deleted and inserted at each junction, giving rise to junctional diversity in addition to recombinational diversity.
PCR, and the percentage of putative malignant cells in the sample should be taken into account when interpreting the result of the assay. For example, a recent study used the well validated BIOMED-2 TCRG primer set\(^4\) to examine 203 biopsies from patients with confirmed late stage mycosis fungoides or Sezary syndrome\(^5\). 180/203 samples tested positive, giving a sensitivity of 89% for this assay. In another study, 59/77 biopsies from patients with a variety of different cutaneous T-cell lymphomas tested positive using the same PCR primer set, giving a sensitivity of 77%.

**False positive results**

One reason for “false positive” findings is simply that benign clonal proliferations can occur – clonality does not necessarily imply malignancy. For example, clonal populations have been reported in cases of lichen planus, pityriasis lichenoides and lichen sclerosus.\(^5\) There have also been reports of clonal expansions in pseudolymphomas, i.e. benign disorders that can histologically simulate cutaneous lymphoma, such as some insect bite reactions and drug eruptions.\(^5\)

In addition to benign clonal expansions, false positives can be seen for assay-related reasons – for example when there is very little amplifiable DNA extracted from the sample due to low cell numbers or DNA degradation. As noted above, if a true clonal population is present, this can lead to a false negative result. However, low amounts of amplifiable DNA can also sometimes produce a false positive result, known as ‘pseudoclonality’. If there is very little initial amplifiable material, one or a few V(D)J segments may start to predominate over the others early in the exponential PCR amplification process. As the reaction continues, these segments can ‘take over’, giving the appearance of a clonal band, or several clonal bands (‘oligoclonality’). Recent studies have examined the problem of false positives in benign skin disorders using the BIOMED-2 TCRG assay. In one study\(^6\), 4/30 pseudolymphomas gave false positive TCRG results due to pseudoclonality (87% specificity), while in another, 11/80 cases of benign inflammatory disease gave false positive TCRG results due to either pseudoclonality or the presence of benign clonal expansions (86% specificity).\(^4\)

**Summary**

Gene rearrangement assays are based on the fact that each B- or T-cell undergoes rearrangement of their immunological receptor genes during maturation. These tests are most useful in biopsies where histopathology and immunophenotype does not give a clear result. As such they are usually requested by histopathologists, after thorough initial investigations have been carried out.

It is important to be aware of the caveats of this type of assay, both biological (i.e. clonality does not necessarily imply malignancy) and technical (false negative and false positive results are possible, particularly when there are low numbers of lymphocytes or the sample is of poor quality). For these reasons, this assay should not be used as a sole indicator of malignancy, and results should always be interpreted in the light of clinical information, histological findings, and immunophenotype.

**References**

1. Figure adapted from Van Dongen JJM et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. Leukemia 2003; 17(12): 2257-2317.