CHAPTER 5: CONCLUSION

This study reports the results of 215 patients with various haematological malignancies. The study has shown that immunophenotyping is a useful as well as a necessary adjunct to conventional methods of diagnosis of leukaemias.

Immunophenotyping identified four major subgroups in ALLs. The majority were C-ALLs (54%) followed by T-ALL (24%). Paediatric patients tend to predominate among Null-ALL, C-ALL and B-ALL, while adults tend to predominate among T-ALL, AML and CLL cases. The distribution of sexes was random among subphenotypes except T-ALL, in which males predominated (82%).

The need to recognize and differentiate the numerous stages of maturation in immune cells is necessary for diagnosis and treatment, and has led researchers to approach the study of these cells from the cellular, protein, RNA to DNA levels.

Morphological study of cells requires a haematopathologist to assign the FAB designation. As this is a subjective approach, experience and time are needed to produce highly trained personnel.
The distinction of cell lineage (myeloid, B and T cells) by morphological means (which is supported by cytochemistry tests) compared with immunophenotyping showed actual discordance in only 5 out of 181 cases (3%). However, the statement "... L2 subtypes include almost most of the T-ALLs..." (Hoffbrand and Pettit, 1988) cannot be closely adhered to because it was shown in our studies that almost the same percentages of L2s were found among C-ALL (42%) and T-ALL (44%).

It is obvious that IP easily identified the sublineages in the B and T cells, and this is important for the first mode of treatment, that is chemotherapy. The immortality of stem cells and the resistance of mature undividing cells to cytotoxics have to be taken into consideration when treatment is considered. Intermediate cells, for example, Null-ALL and B-ALL from C-ALL need to be identified separately. For diagnostic purposes, a minimum of three markers, B4, Calla and SmIg, readily identifies these sublineages. Similarly, T cells can also be categorized and differentiated by using another three markers, T2, T11 and T3 (to identify categories I, II and VI, respectively) or T2, T4/T8 and T3 (to identify categories I, IV and VI, respectively), depending on which subgroup turns out to have a better prognosis. Since leukaemic burden is also indicative of prognosis, a look at the total white blood cell count in

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tive of prognosis, a look at the total white blood cell count in Table 12a showed that among category II, the majority (60%) of cases had a TWBC of less than $9 \times 10^9$ cells/L, samples in category IV had more than $30 \times 10^9$ cells/L of white blood cells while category V consisted of cases with TWBC of $9 \times 10^9$ cells/L. Therefore, in the order of improving prognosis, they are from categories V, IV to II.

In view of the fact that cells express antigens weakly or strongly, these should also be taken into consideration because they represent different stages of maturation.

By the use of surface markers, a homogenous population of cells was uncovered among M1 and M2 designated samples. Unless more markers are used it shows that IP is unable to differentiate M1 from M2 cells. Furthermore, another subgroup, M0, also has the same phenotype as M1 and M2 (HLA-"DR+, My7+ and My9+). This group cannot be detected by cytochemistry tests because it is myeloperoxidase negative, nor differentiated by morphology. In this instance it appears that a combination of all three methods is required to identify these three subgroups.

IP appeared to be better at identifying M3 and M7 subgroups. Pathologists identified only two M3 cases when there were at least five others with a similar phenotype. M7 cells expressed intermediate levels of HLA-DR and were negative for
My7, My9, My4 and Mol. They expressed the megakaryoblastic markers. They are also myeloperoxidase negative, thus they will have to be differentiated from the M0 subgroup.

Among the M5, however, a heterogeneous population was detected by surface markers. By phenotype they could be assigned to the other subgroups including M4.

Cell cultures provide us with a means to see living cells in action. Normal functions and activities of a particular group of cells may be identified while the progression of maturation stages observed in *in vivo* and *in vitro* studies. The natural environment of immune cells, however, is a lot more complex than expected. As cells, they are largely dependent on external signals which determine their next course in life. The fact that lymphocytes, for example, are given birth, nurtured and mature in totally different conditions already hints at the many determining factors they must encounter. Furthermore, mature cells respond differently from their immature counterpart to the same activator, which may command termination of the cells' lives or that they be destined as sole guardians of a specific region in the living organism. The indiscriminate usage of various known growth factors these days may now be a matter of discontent because it disallows proper comparison between studies.

*Cells are of course stimulated by receptors expressed on*
their surface. Special receptors distinguish one cell lineage from another and they are used as markers to identify the group. A combination of markers allows a particular maturation stage to be made known. Unfortunately, the majority of surface markers were found to be non-specific, harbouring certain levels of infidelity. Whether the expression of a marker on another cell type has a function or is just an aberrant occurrence on a normal cell has yet to be known simply because the function of these receptors is not all known. At present, it serves to complicate identification of a cell type, not so much in leukaemic cases but more so on lymphoma cases in which the origin of the cancer cell is at best vague. Which is why the origin and the ability of cells to migrate from one part of the body to another should be researched.

The discovery of rearranging elements at the DNA level in lymphocytes paved the way for the molecular approach, enabling these cells to be studied at a different angle. Only the T and B lymphocytes were found to possess this unique system, which is similar in basic structure but differing in end product and detail. The Southern blot technique made it possible to observe rearrangements as a whole and identify the rearranging system. Changes in one system which result in changes in another system can be observed, for example, loss of the 6.0, 3.0 and 1.6/1.4 kb bands in the TCR delta system resulted in a similar loss of bands in the TCR alpha
system. A monoclonal population of an abnormal cell was easily distinguished from a polyclonal population of normal cells by way of the different pattern of restriction fragments revealed on the blot.

Results here showed a sequence in rearrangements within individual TCR genes and also between the TCRs. The relatively extensive rearrangements seen in delta genes here suggest that more immature cells probably exist which will need to be discovered and placed earlier to cells in category I. Sequence in rearrangements was especially observed in the gamma gene, where transition from germline to C1 and finally C2 usage among more mature samples were seen. In the beta gene, early cells had C1 rearrangement on one allele and C2 rearrangement on the other, while more mature cells rearranged to C2 on both alleles. It would be interesting to see the type of beta gene rearrangement pattern in more immature cells. The lack of rearrangements among the alpha genes, however, suggests alternative probes will need to be used so that more activities may be observed. The existence of two constant regions in the gamma and beta systems made it possible to observe the sequence of changes. In the other two systems, more than one probe, situated at various points along the gene, will be required.

It was soon found that rearrangements were not restricted to only lymphocytes or the cell’s (B or T) own system but occurred quite rampantly on the other lymphocyte (T or B) as
well as myelo-monocytic lineage cells. This is similar to
the non-specific expression of surface markers. Immature
cells appeared more rearranged for the 'other' system than
do mature cells, further complicating the matter. There is
no reason why changes at the DNA level should be specific as
all cells carry the same genes and have the same basic
metabolic system, for example. As aberrant surface marker
expressions are now found to be phenotypes of normal cells,
more research in gene analysis will have to be done to 'legalize'
the existence of non-specificity in gene rearrangements.

The Southern blot technique, however, is labour intensive
and time consuming and the usage of radiolabelled probes is
potentially harmful. Interpretation of results is also
tedious as it can become quite subjective. With PCR-based
DNA/RNA analysis, DNA research would be made easier.

The PCR technique was also applied to detect minor (abnor-
mal) populations, by using specific probes. The PCR method
is valuable for diagnostic purposes and is hoped to bring us
closer to understanding the non-specific rearrangement of
genes. However, known primers have to be available and
observations are limited to tailored conditions.

RNAs were isolated by using the Northern blot technique to
find out which of the DNAs were actually transcribed.
Results showed transcription was much more specific since only RNAs of one lineage were detected. However, within a single lineage, RNAs were not transcribed as expected at the various maturation stages. Northern blot also presents a problem of its own as it is a sensitive method requiring high sterility which has to be maintained throughout and which the main contaminant is the person him/herself. Monoclonal antibodies against the protein actually expressed on a cell will give a more direct look at clonality.

It is obvious no single approach will give a complete picture. The best way is to combine at least two studies for the whole story.