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**PROLIFERATING KI67 EXPRESSING B-CELLS ASSOCIATE WITH**

**CD4+PD1+ T-CELLS IN MARGINAL ZONE LYMPHOMA**

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**Background:** Specific microbial antigens have been implicated in the development

and maintenance of several types of marginal zone lymphoma suggesting

that an abnormal immune response is essential for driving B-cell proliferation.

Reasoning that T-cell stimulation, especially from CD4+ T-cells, might

make important contributions to promoting B-cell proliferation we carried out a

detailed analysis of infiltrating T-cells in 8 cases of nodal marginal zone lymphoma.

**Aims:** The aim of the study was to undertake the first detailed study of T-cell

subsets in marginal zone lymphoma in order to determine the relationship of

individual subsets to proliferating lymphoma B-cells.

**Methods:** We carried out multiplex immunohistochemistry and validated the

results to show that, for the combinations of antibodies employed, there was

firstly no reduction in intensity after several rounds of staining and destaining

and secondly that there was no significant carry over from one round to the

next. The stained slides were scanned and, utilising a custom macro written

for ImageJ software, we enumerated CD4+ T-cell subsets (TH1- CD4+TBET+,

Treg - CD4+FoxP3+, follicular helper (Tfh) - CD4+PD1+ and follicular regulatory

(Tfr) - CD4+FoxP3+PD1+).

**Results:** In all cases CD4+,T-cells constituted a major portion of infiltrating Tcells,

mean 39.8% (range 13.5 to 70.3%). There were, however, large differences

in the CD4+ T-cell subset composition; Tfh cells varied from 2.5 to 36%

of all CD4+,T-cells,whilst Tregs accounted for 2.7 to 24.7%. We also compared

architecture of T-cell infiltration across cases and found that T-cells were not

homogenously distributed and that CD4+PD1+ cell clusters could show some

association or no association with CD4+FoxP3+ clusters and, in one case,

repulsion from CD4+FoxP3+ clusters. In order to quantitate the associations

we carried out Pearson correlations. For comparison normal tonsil showed a

Pearson correlation of -0.4 *i.e.* no overalp, between CD4+PD1+ and

CD4+FoxP3+ cells whereas there were varying degrees of association (range

0.3 to 0.8) for the lymphoma samples. By contrast proliferating Ki67+ lymphoma

B-cells associated with CD4+PD1+ cell clusters (Pearson 0.1 to 0.6) whatever

the relation to CD4+PD1+ cells to CD4+FoxP3+ cells. To confirm this result we

used an alternative method to analyse clustering (the Morisita index). This produced

similar results with normal tonsil having a Morisita index of 0.2 for

CD4+PD1+ and CD4+FoxP3+ whereas lymphoma samples showed higher

degrees of association (range 0.3 to 0.9). The Morisita index also confirmed

association between proliferating B-cells and CD4+PD1+ cells; normal tonsil

0.8 and lymphoma (0.4 to 0.8).

**Summary/Conclusions:** Collectively our data suggests an unsuspected association

between CD4+PD1+ T-cells and proliferating lymphoma B-cells in marginal

zone lymphoma.