Lymphocyte subset analysis by flow cytometry in Beagle dogs

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Introduction
Lymphocyte subset analysis is an immunotoxicologic endpoint commonly included in rodent studies, but there is a lack of data in the dog. The aim of the present study was: (i) to start establishing the normal range of lymphocyte subsets in control male and female Beagle dogs; and (ii) to evaluate changes in lymphocyte subsets in Beagle dogs treated with either cyclophosphamide or cyclosporine.

Materials and methods

Lymphocyte subset analysis
The analysis of blood lymphocyte subpopulations with a Navios 10-color flow cytometer was validated for linearity and precision, and absence of non-specific binding, using the PE-LSM 11425 antibody for B cells, APC-LSM 8.358 for total T cells, PE-LSM 12.125 for CD4+ T cells, and FITC-LSM 1140 for CD8+ T cells (BD Pharmingen). CD4+ T cells were also analyzed with the RPE-YKIX302.9 antibody, CD8+ T cells with the AF-647-YCATE55.9 antibody and CD3+ T cells with the FITC-CA17.2A12 antibody (AbD Serotec).

Treatment
Groups of 3 male and 3 female Beagle dogs were treated orally for 4 weeks with 2 mg/kg cyclophosphamide on 2 consecutive days each week, or 25 mg/kg cyclosporine daily, or the same volume of drinking water daily. Lymphocyte subsets were analyzed twice at a 7-day interval before the start of treatment, and then on days +1, +11, +18 and +28 using the validated assay with the BD Pharmingen antibody combinations.

Results

Lymphocyte subsets in control Beagle dogs
There was no non-specific binding. Intra-day variability measured in 3 control males was within the study acceptance criteria: total T = B lymphocytes = 100% ± 25% (NK cells were not identified); (CD4+ T) + (CD8+ T) lymphocytes = total T lymphocytes ± 20%. Intra-individual inter-day variability was found to be low over a 40-day period (figure 1).

The comparison of values (Bland-Altman correlation plot) obtained for CD4+ T helper lymphocyte counts using either the APC-LSM 8.358/PE-LSM 12125 (BD Pharmingen) or the FITC-CA17.2A12/RPE-YKIX302.9 (AbD Serotec) antibody combinations showed identical results, while the FITC-CA17.2A12/AF647-YCATE55.9 combination (AbD Serotec) resulted in a constant mean bias of +0.3 G/L compared to the APC-LSM 8.358/FITC-LSM 1140 antibody combination (BD Pharmingen) for CD8+ T cytotoxic lymphocyte counts.

Values of lymphocyte subsets have subsequently been obtained in 37 males and 48 females Beagle dogs (Table 1, figure 2) using the BD Pharmingen antibody combinations.

<table>
<thead>
<tr>
<th>Lymphocyte subset</th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td>B lymphocytes</td>
<td>0.57 ± 0.17 G/L</td>
<td>0.55 ± 0.20 G/L</td>
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<tr>
<td>Total T lymphocytes</td>
<td>1.86 ± 0.43 G/L</td>
<td>1.99 ± 0.47 G/L</td>
</tr>
<tr>
<td>CD4+ T helper lymphocytes</td>
<td>1.19 ± 0.33 G/L</td>
<td>1.31 ± 0.36 G/L</td>
</tr>
<tr>
<td>CD8+ T cytotoxic lymphocytes</td>
<td>0.39 ± 0.10 G/L</td>
<td>0.41 ± 0.10 G/L</td>
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</tbody>
</table>

Table 1: Values of B, total T, CD4+ T helper, and CD8+ T cytotoxic lymphocytes obtained in 37 male and 48 female control Beagle dogs (Mean ± SD).

Figure 1. Intra-individual inter-day variability. Males in blue; Females in green.

Figure 2. Normal range of blood lymphocyte subsets in control Beagle dogs.

Lymphocyte subsets in cyclosporine- or cyclophosphamide-treated Beagle dogs
No effects of cyclosporine were observed on any lymphocyte subset. In contrast, cyclophosphamide markedly decreased lymphocyte counts from day +11 until the end of the treatment period and this finding correlated with decreases in B lymphocytes (figure 3).

Figure 3. Effects of cyclosporine and cyclophosphamide on lymphocyte subsets.

Conclusion
These results show that lymphocyte subset analysis can be easily included in regulatory immunotoxicity studies in Beagle dogs. To enhance the reliability of the presented range of normal values, lymphocyte subset analysis is being performed in additional control male and female animals.

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