Supplementary Table 1

Correlation analysis between the number of specific cell types placed in culture and e*x vivo* IL-2 production following ConA stimulation

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Correlation between IL-2 production and lymphocyte subset (pg/x104 cells¥) | | | | | |
|  | All groups | | ChS | | ChD | |
|  | rs‡ | P-value | rs‡ | P-value | rs‡ | P-value |
| CD4+CD25+ | -0.007 | 0.982 | -0.317 | 0.406 | 0.600 | 0.285 |
| CD8+CD25+ | 0.187 | 0.522 | 0.333 | 0.381 | 0.300 | 0.624 |
| CD4+CD71+ | 0.433 | 0.122 | 0.600 | 0.088 | 0.600 | 0.285 |
| CD8+CD71+ | -0.108 | 0.714 | -0.183 | 0.637 | -0.100 | 0.873 |

CD, cluster of differentiation;

† The total number of immune cells was calculated by multiplying the % of immune cell phenotype in the spleen by the total number of splenocytes isolated (x106).

‡ Spearman’s correlation coefficient used to assess correlation between lymphocyte population and IL-2 production.

\* indicates mean within a row that is significantly different from ChS group (P<0.05).

Supplementary Table 2

*Ex vivo* IL-2 production by the number of specific cell types placed in culture following ConA stimulation

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | ChS | | |  | ChD | |
| n† | 10 | | |  | 7 | |
|  | Mean | SEM | |  | Mean | SEM |
|  | IL-2 production (pg/x104 cells of lymphocyte subset‡) | | | | | |
| CD4+CD25+ | 1043 | | 154 |  | 702\* | 70 |
| CD8+CD25+ | 1113 | | 160 |  | 603 | 95 |
| CD4+CD71+ | 542 | | 44 |  | 362 | 38 |
| CD8+CD71+ | 407 | | 62 |  | 197 | 26 |
| CD4+CD25+ and CD8+CD25+ | 523 | | 74 |  | 323\* | 41 |
| CD4+CD71+ and CD8+CD71+ | 228 | | 26 |  | 127 | 15 |

CD, cluster of differentiation;

† n refers to the number of dams as they are the experimental unit. This includes two offspring pooled to obtain a measure for each dam, with measurements from each offspring conducted in duplicate

‡ To express cytokine response by specific lymphocyte population, the % of lymphocyte population was multiplied by 1.25x106 (number of total cells added to culture). Then IL-2 production (pg/ml) was divided by the number of lymphocytes added to culture to express the amount of IL-2 in the media per a specific lymphocyte subset

\* indicates mean within a row that is significantly different from ChS group (P<0.05)

Supplementary Figure Legends

SUPPLEMENTARY FIGURE 1. Examples of representative dot plots for gating strategies used in flow cytometry analysis of splenocytes (A-D representing one individual animal)

SUPPLEMENTARY FIGURE 2. Examples of representative dot plots for isotype controls used in flow cytometry analysis including the gating strategy (A), and histograms of isotype controls FITC (B), PE (C) and PerCP (D)

SUPPLEMENTARY FIGURE 3. Representative dot plots for unstained splenocytes (A) and isotype controls (B-E) used in flow cytometry analysis