Isolation of CD8a+ T cells to high purity from postnatal mice using MACS® Technology

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Introduction

To achieve an accurate, unbiased analysis or to establish a homogenous culture of a distinct cell type, it is often essential to use a highly pure cell population. CD8a+ cells can be isolated to high purities from adult mice spleens using CD8a (Ly-2) MicroBeads, mouse from Miltenyi Biotec. The percentage of CD8a+ cells among splenocytes from adult mice ranges between 15 and 18%,¹ which is optimal for isolation with CD8a (Ly-2) MicroBeads. However, in spleens from postnatal mice the percentage of CD8a+ cells is significantly lower, in the range of 3%,¹ and we could not achieve sufficiently high purities of CD8a+ cells in this case.

Based on MACS® Technology, we developed a protocol for the effective isolation of CD8a+ cells from postnatal mouse spleen, and achieved CD8a+ cell purities of greater than 90%.

Materials and methods

Spleenic single-cell suspensions from postnatal (P12–P15) C57BL/6N and BALB/c mice were prepared as described.¹ CD8a+ cells were isolated in a three-step process. First, CD45R+ B cells were depleted from the splenic cell suspension using CD45R (B220) MicroBeads and an LD Column (Miltenyi Biotec) according to the manufacturer’s instructions. The negative, unlabeled fraction from this separation step was used for subsequent positive selection of CD8a+ cells with CD8a (Ly-2) MicroBeads (Miltenyi Biotec) and an LD or MS Column according to the manufacturer’s instructions. To further increase the purity, the positively selected, labeled CD8a+ cells were applied to a second, freshly prepared LS or MS Column. The process is illustrated in figure 1. Cell separation results were evaluated by flow cytometry. Data shown are means and standard errors of the means from at least five different experiments.

Results

Using CD8a (Ly-2) MicroBeads as a sole means for the isolation of CD8a+ cells from postnatal mouse spleens, we obtained purities of about 66%. While being sufficient for some applications, this purity is too low for many molecular analyses and cell culture techniques, for example. CD8a MicroBeads are designed for use with cell suspensions containing between 7% and 15% CD8a+ cells, which is in the range of cell suspensions from adult mouse spleen.¹ We found that cell suspensions from postnatal spleens, however, contained significantly lower proportions of CD8a+ cells (3%, ref. 1) compared to adult spleens. The low percentage in postnatal spleen most likely led to the suboptimal purity of CD8a+ cells after enrichment, and we reasoned that depletion of a large portion of unwanted cells would raise the proportion of CD8a+ cells to the optimal range for positive selection with CD8a MicroBeads. We found that CD45R+ cells were present in large numbers in postnatal spleen (28–31% of total living cells, not shown). CD45R is considered a pan B cell marker, but is also expressed on activated T cells, dendritic cells, and other antigen-presenting cells.² However, in postnatal spleens the frequency of activated CD8a+ T cells should be low. Therefore, depletion of CD45R+ cells from the splenic cell suspension should not decrease the CD8a+ cell population to a great extent, and we decided to deplete CD45R+ cells to pre-enrich CD8a+ cells prior to positive selection. The depletion led to a significant increase in the percentage of CD8a+ cells from 3% to 7%. Following positive selection, the purity of CD8a+ cells was 73.2±10% for BALB/c and 57.8±12.2% for C57BL/6N mice (fig. 2). To further increase the purity, we applied the positive cell fraction to a second column, resulting in CD8a+ cell purities of 96.0±2.0% (BALB/c) and 92.3±3.2% (C57BL/6N). Overall, this strategy increased CD8a+ cell purity by approximately 30% compared to positive selection of CD8a+ cells alone.
**Conclusion**

We developed an optimized procedure for the isolation of CD8a+ cells from spleens of postnatal BALB/c and C57BL/6N mice. Using CD45R (B220) MicroBeads and CD8a (Ly-2) MicroBeads, we achieved purities of greater than 90%. This protocol is particularly beneficial for applications where high purities of postnatal CD8a+ cells are required.

**References**
