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Determining the Role of CD5 in regulatory T Cell Function

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Introduction

Regulatory T cells (Tregs) inhibit the immune response and play a key role in preventing autoimmunity. Unfortunately, Tregs also often prevent an effective immunological response against cancer. CD5 is a T cell surface protein known to regulate the threshold for T cell activation. CD5 is known to influence Treg development, but it is unclear exactly how CD5 impacts the function of Tregs.

Past studies have indicated that mice lacking CD5, known as CD5 knockouts, produce more Tregs than wild type mice (1). It is thought that Tregs from these CD5 knockouts may be more effective in preventing T cells from being activated. Studies have confirmed an increase in Tregs in CD5 knockouts while noting that typical Tregs contain high levels of CD5 (2). Recent studies have found that high expression of CD5 prevents processes that normally stop the development of Tregs (3). It seems contradictory that the absence of CD5 would lead to an increase in Tregs when it is evident that CD5 plays a role in encouraging Treg activation. The actual effect of CD5 of Treg suppressive capacity requires further elucidation. Our goal was to determine how effective CD5 deficient Tregs are in suppressing an immune response in comparison to Tregs with CD5.

Methodology

To clarify how CD5 levels alter Treg function, we explored how Tregs in CD5 knockout mice differ in their ability to suppress the immune system. The first objective was to reliably isolate CD4+CD25- effector T cells and CD4+CD25+ Tregs from the spleens of wild type and CD5 knockout mice. The sorting was accomplished via negative and then positive selection MACS column sorting which allows the CD25+ Treg fraction to be separated from the effector T cells for reintroduction in vitro. The purity of these sorts was evaluated by flow cytometry and found to be adequate for our suppression assay.

The CD5 knockout Tregs and the wild type Tregs were introduced in serial dilution to aliquots of $3 \times 10^4$ cells per 50 microliters of media was optimal for avoiding prohibitive overcrowding of proliferating cells. We also determined that ratio of two-to-one CD3/CD28 microbeads per cell provided the best conditions for T cell proliferation.

Results

Effective co-culture and proliferation of Tregs and CD4+ T cells proved initially difficult. Repeated trials demonstrated that a concentration of $3 \times 10^4$ cells per 50 microliters of media was optimal for avoiding prohibitive overcrowding of proliferating cells. We also determined that ratio of two-to-one CD3/CD28 microbeads per cell provided the best conditions for T cell proliferation.
Once the proliferation and suppression assay technique was refined the influence of CD5 knockout and wild type Tregs on effector T cells could be quantified. Preliminary results indicate that CD5 knockout Tregs have a significantly reduced suppressive capacity in relation to wild type Tregs. CD4+ T cells saw a 28.5-12.6 percent reduction in cell division in the presence of wild type Tregs while CD5 knockout Tregs caused only a 6.8 to 0.7 percent reduction after a 72-hour period. The range in proliferation was determined by serial dilution of the Tregs co-cultured with the effector T cells.

Discussion and Conclusion

Our results indicate that there appears to be a marked reduction of suppressive capacity in Tregs lacking CD5. It should be noted that our assay has only been successfully performed twice and so these results are only preliminary until repeated trials can be done to confirm the statistical significance of this difference in suppressive ability. If this pattern holds true it would provide evidence that CD5 plays an important role in Treg activity but indicate that the increase in Treg numbers in CD5 deficient mice does not necessarily correlate to increased suppressive activity.

Once results from the suppression assays are shown to be consistent we hope to move forward with our plan to determine if CD5 plays a key role in Treg recruitment by tumors by performing a cytotoxicity assay of the T cell response against in vitro tumors with our selected Tregs. This should provide a much more useful metric of the degree to which CD5 expression influences the immune response in the tumor microenvironment.

References

