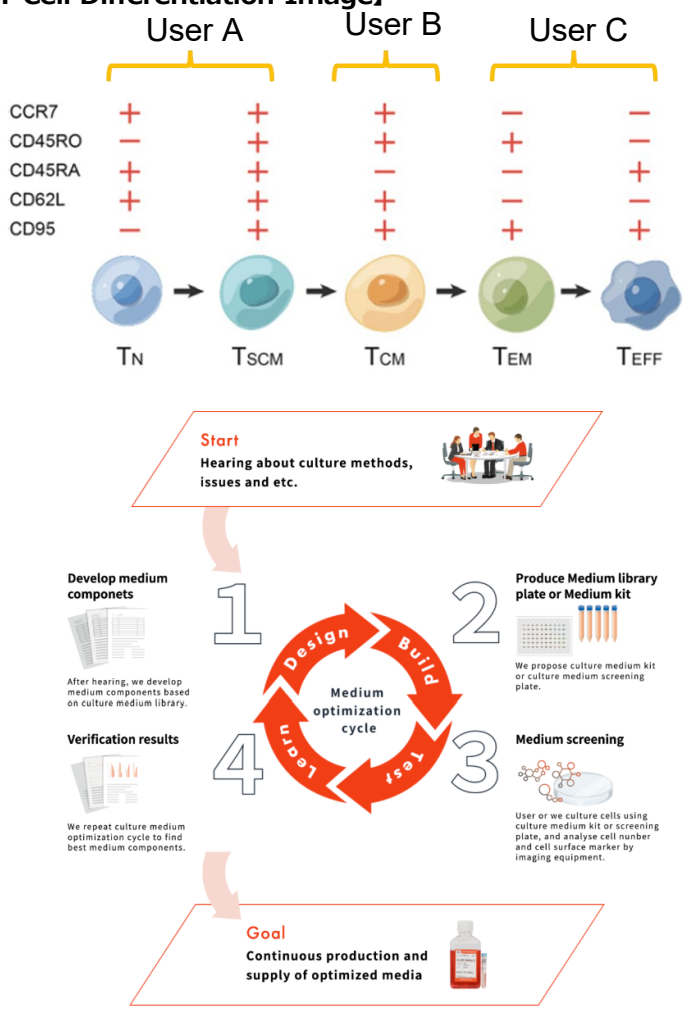
Our Concept for T Cell Culture Medium

Myoridge
Application No.8

Background, Concept 1

【T Cell Differentiation Image】



Cell culture media, often a single type, are frequently used across both basic research and clinical development. It is highly likely that this situation often prevents individual cells from fully expressing their intrinsic capabilities, especially in primary cell studies. For example, while the desired final product may be a T cell population where the undifferentiated state is maintained, or a population with moderate differentiation, or one in the effector stage, a single type of medium makes it difficult to address these diverse requirements.

To solve the above-mentioned issues, we provide the T-cell culture medium requested by each user by iterating through the medium optimization cycle shown on the left. Our T cell culture medium is fundamentally serum-free or xeno-free, but can be modified according to user requests.

https://doi.org/10.1186/s40364-022-00434-9

Background, Concept 2

Culture media are composed of a basal medium and supplements, but an optimal basal medium is required to maximize the performance of each supplement. For T cell culture, RPMI 1640 is commonly used, but our studies have also identified combinations where the performance of various supplements is enhanced when using a basal medium other than RPMI 1640.

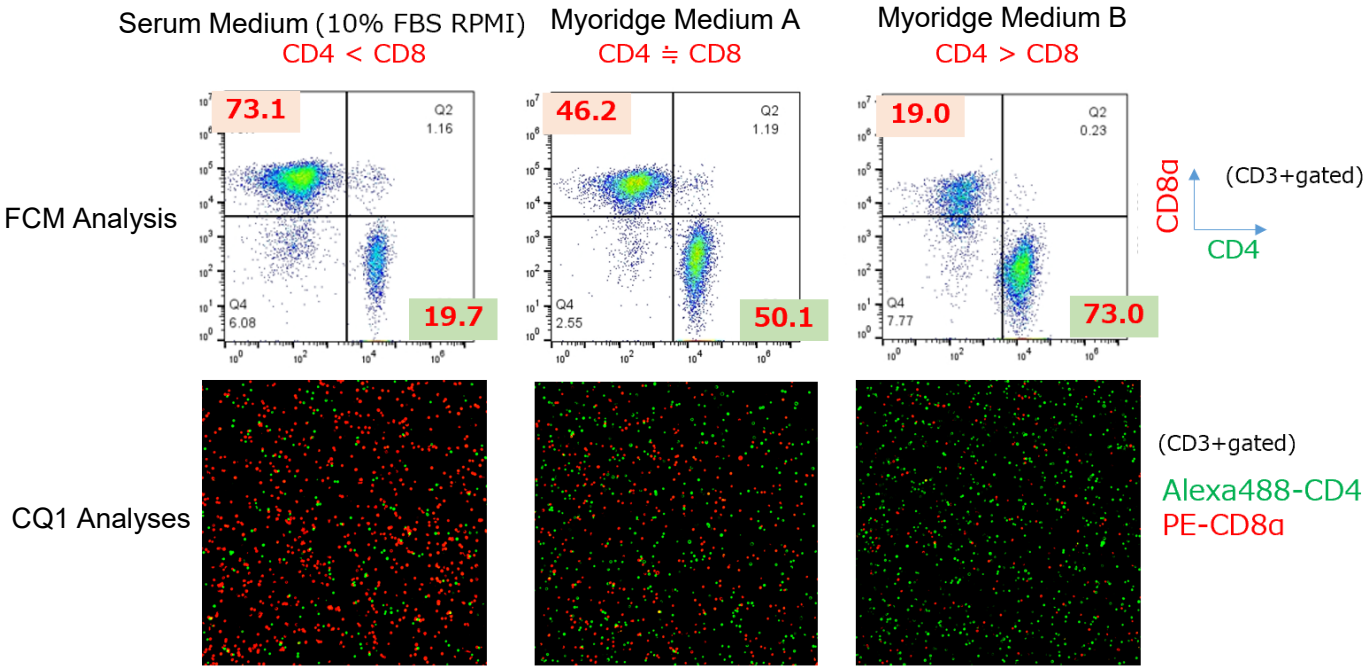
Cell Phenotype Control using Our T cell Culture Medium (CD4/CD8 Ratio, Internal Study)

Myoridge
Application No.8

Methods and Results 1

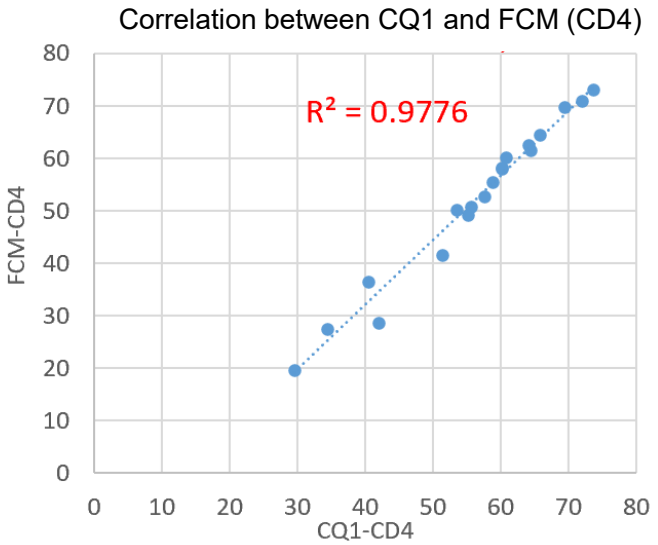
(Methods) 1 × 10^6 PBMCs were cultured for 10 days with OKT3/IL-2 (10ng/ml). Subsequently, the CD4/CD8 ratio was measured using FCM or CQ1 (Yokogawa Electric Corporation)

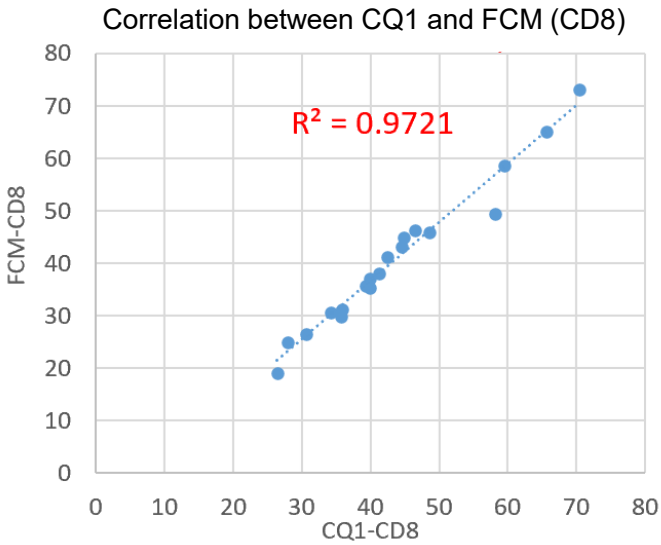
(Results) The CD4/CD8 ratio varied depending on the culture medium used, even when the same PBMCs and the same stimulation were employed. The proliferation rate was comparable between the control medium (10% FBS RPMI) and our Media A and B.



(Methods) PBMCs were cultured in 20 different media with OKT3/IL-2 (10 ng/mL) for 10 days. Subsequently, the CD4/CD8 ratio was measured using FCM or CQ1.

(Results) The CD4/CD8 ratio showed a high correlation between the FCM and CQ1 analyses. It was suggested that CQ1 is suitable for small-scale screening analysis of individual PBMC samples because it requires approximately 1 x 10^3 cells, whereas FCM typically requires around 1 x 10^5 cells.





Cell Phenotype Control using Our T cell Culture Medium (CAR-T Cell Induction, External Institution Study)

Myoridge
Application No.8

Methods and Results 2

(Background) Myoridge and Mitsubishi Chemical Corporation are jointly developing a T cell culture medium. In this investigation, several candidate media emerged that showed IL-2 concentration-dependent proliferation and exhibited juvenile characteristics in small-scale T cell cultures (24-well/6-well plates). Based on these results, we provided multiple media candidates to AGC Inc. (hereafter, "AGC") for evaluation in their CAR-T cell induction system.

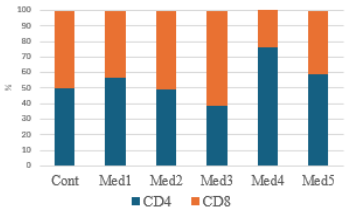
(Methods) Using PBMCs that have previously demonstrated success within AGC, and following a specified protocol, CAR-T cell induction was performed. In addition to the Control (Cont), media candidates designated Med1 to Med5 (which are analogous to Our Company's Medium A) were used. Subsequently, proliferation, CAR positivity rate, and FCM analysis were evaluated.

(Results) Compared to the Control (Cont) medium, Med1 showed the highest proliferation among the Med1-5 candidates. Its surface antigen pattern, however, was dominated by the CM/EFF (Central Memory/Effector Memory) population, similar to the Cont medium. On the other hand, Med4 and Med5 detected a large population of SCM/CM (Stem Cell Memory/Central Memory) cells. Notably, Med5 showed proliferation comparable to the Cont medium.

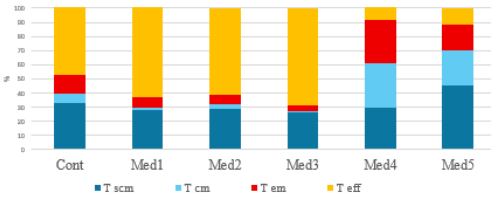
(Summary)　The six media types can be broadly categorized into Cont, Med1-3, and Med4-5. Med5 was determined to be the most superior in terms of both proliferation and juvenile characteristics. Conversely, Med1 showed results suggesting high proliferation and potential for short-term anti-tumor efficacy.

	cont	Med1	Med2	Med3	Med4	Med5
Total Cell Proliferation Rate (%)	640	789	572	519	537	641
CAR Positivity Rate (%)	28	10	15	12	18	22

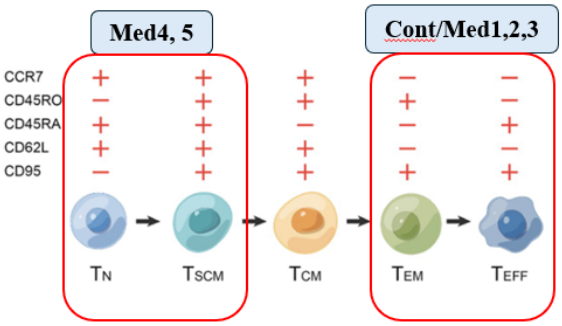
Post-Culture CD4/CD8 Ratio



SCM/CM/EM/EFF Subsets Ratio



Key Population Images



(AGC Inc. Contact URL)
URL: https://www.agc.com/contact/index.html#product5

Summary

It was suggested that optimizing the medium composition allows for the induction of phenotypes requested by individual users. This current investigation utilized PBMC samples from healthy donors. The plan is to next examine whether similar phenotype control is possible even under unfavorable conditions, such as with poorly proliferating samples.